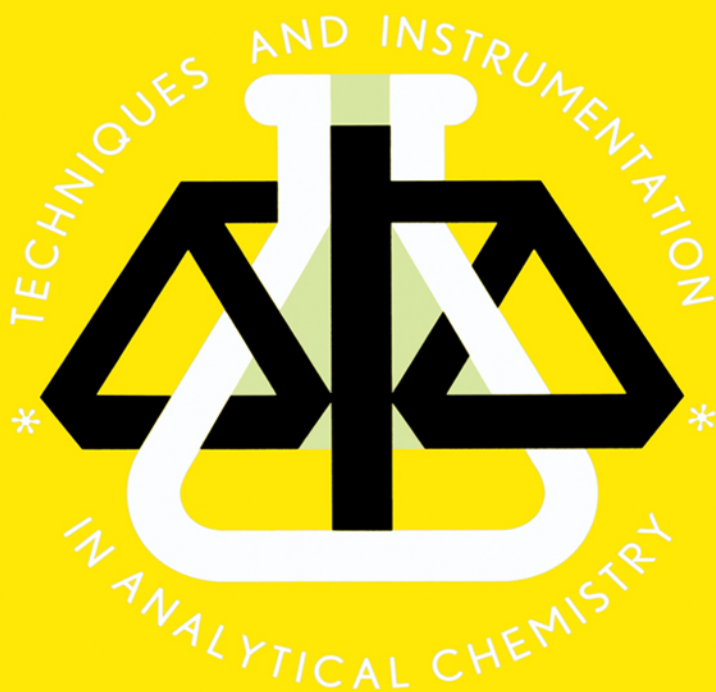


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**INTERLABORATORY STUDIES
AND CERTIFIED REFERENCE
MATERIALS FOR
ENVIRONMENTAL ANALYSIS
THE BCR APPROACH**

**PH. QUEVAUVILLER
E.A. MAIER**

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THE BCR APPROACH
By Ph. Quevauviller and E.A. Maier

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This book is dedicated to Ben Griepink and Herbert Muntau.
Without their joint efforts and ‘struggle for quality’, none of the
materials described in this book would ever have been produced.

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Preface

Reference materials and interlaboratory studies are two of the major tools that are available to analytical laboratories to monitor the performance of their work. In several branches of the economic sector, in particular industry, these tools have been in use for several decades and are driven by the market. For other fields, like monitoring of the environment, health and safety services, these tools are often supported by public funding until the potential market reaches a sufficient size to become self-sufficient. Since 1973, BCR and the successive research and development programmes of the European Commission have recognised this situation and have funded a wide range of projects to set up interlaboratory studies and develop/produce Certified Reference Materials (CRMs). The authors had the chance to be involved, over several years, in these activities, in particular in the environment, food and health sectors. It is their experience that is reflected in this book. It should be noted that a single book can hardly contain a complete view of activities, over such a wide sector, covered by reference material applications; some readers might hence regret that their field of interest has not, or not sufficiently, been treated. While the authors have aimed to cover the general aspects of the preparation and certification of environmental CRMs, the case studies are very much oriented towards BCR reference materials produced within the last 15 years. Additional information on CRMs available from other producers worldwide can be found through references quoted in the various chapters.

The book is composed of twelve chapters, of which the first five focus on detailed descriptions of quality assurance (Chapters 1 and 2) and on the use, production and certification of reference materials (Chapters 3, 4 and 5). A particular effort has been made to present in more detail those studies and materials linked to microbiological measurements. This special emphasis is intended to fill a gap in the literature, where CRM and interlaboratory studies for microbiology are too often absent. Chapters 6 to 11 provide comprehensive information on more than 70 CRMs produced by BCR during the period 1983–98, covering various matrices such as plant materials (Chapter 6), biological materials (Chapter 7), waters (Chapter 8), sediments (Chapter 9), soils and sludges (Chapter 10) and other materials (coals, ashes and dusts, Chapter 11) and a wide variety of parameters (e.g. major and trace elements, polychlorinated biphenyls, polyaromatic hydrocarbons, organochlorine pesticides, chemical forms of As, Hg, Pb, Se and Sn, and microbiological parameters). Finally, Chapter 12 describes principles and organisational aspects of interlaboratory studies, with case studies illustrating improvement schemes and proficiency-testing schemes.

The knowledge summarised in this book is the result of extensive discussions with many experts who have contributed to BCR-certification programmes. More than 300

laboratories from 18 European countries (EU and Associated states) took part in the projects described here. Contributions from these laboratories are gratefully acknowledged, in particular from the coordinators and their teams who actively participated in the organisation of the certifications. The sections on CRM and intercomparisons in microbiology are taken from the many projects and activities with the team of A.H. Havelaar, P. In't Veld and K.A. Mooijman (RIVN) with whom we would also like to associate the team of J.M. Delattre (IPL).

Many thanks are due to the BCR team of the Institute of Reference Materials and Measurements of the European Commission for a fruitful cooperation, and in particular to Jean Pauwels and Gerard Kramer who have provided us with many of the materials (graphs, photographs) which illustrate this book. We would like to thank also Ellen Michel and Emmanuel Lemoine for their help in producing the manuscript.

The production of this book would not have been possible without the help of several of our predecessors and colleagues. We would like to thank them for their continuous support and the fruitful discussions we shared, namely Elisabeth Colinet, Achim Boenke and Stefaan Vandendriessche. Special thanks go to Ben Griepink and Herbert Muntau, who are both pioneers in the certification of environmental RMs and acted as our 'scientific godfathers'.

Philippe Quevauviller and Eddie Maier

Foreword

In the late 1960s the Joint Research Centre of the Commission of the European Community decided to revise its research programmes, then entirely devoted to nuclear research, and to venture into other fields of activities of European relevance. One of the options, amongst others, was environmental research — not a big issue in those times.

Following some years of preliminary work, in 1972 the Joint Research Centre adopted its first ‘Protection of the Environment Programme’. It consisted primarily of analytical studies of a rather restricted number of environmental contaminants such as sulphur dioxide, mercury, cadmium, lead and a few other compounds in the major environmental compartments. The aim was to establish contaminant inventories, investigate transport routes and assess the final fate of these substances in the environment.

It soon became apparent that the extraordinary variability of contaminants in the environmental compartments under study required the analysis of correspondingly high numbers of samples for a correct description of widely varying metal concentrations in many different matrices. These needs were naturally directly related to the availability of suitable instrumental analytical techniques, and to quality-control tools such as certified reference materials for data validation, and in some cases, for example XRF, for calibration.

In those days, there were just two sources of environmental reference materials available worldwide: the unique Bowen’s Kale, truly the fruit of veritable pioneering efforts by a single scientist, and the NBS Orchard Leaves — the latter a spin-off of the former.

The shockwaves of a lecture, presented by Prof. Guenther Tölg to the Annual Meeting of the Gesellschaft Deutscher Chemiker, entitled ‘Spurenanalytik der Elemente — Exakte Wissenschaft oder Zahlenlotto?’ — which stirred up the scientific community considerably, and helped greatly to convince the management of the Joint Research Centre that the Commission should build up a European reference material production process. In 1972, the Bureau Communautaire de Référence was set up in Brussels under the responsibility of DG XII, the Commission’s Directorate for Science, Research and Development, with a budget of 1.9 m écus and the tasks of acting as a secretariat for evaluating the needs for CRMs, organising and coordinating the analytical certification exercises and distributing the produced CRMs.

Contemporaneously, the Joint Research Centre established the METRE Programme (Mesures, Etalons et Techniques de Référence) in 1972 in support of its experimental activities. Both BCR and METRE focused, at the beginning, on industrial reference materials such as cokes and non-ferrous metal ores, but in 1973 METRE started with

the preparation of two series of six sediment and aquatic plant reference materials. This was followed in 1974 by an agreement with the BCR to establish a working party for the certification of a series of trace elements in environmental reference materials produced at the JRC Ispra. The very fruitful cooperation between the JRC Ispra and the BCR, which continues today, enabled the production over the years of more than 60 certified reference materials and many materials for pre-certification feasibility studies. This cooperation was complemented by the JRC establishment at Geel (Central Bureau for Nuclear Measurements, CBNM) which excelled in those years in the production of ultra-pure metal CRMs, and by the JRC Petten where pure compounds, PAHs first of all, were produced for a number of years.

The success of the early BCR–JRC cooperation, especially in the field of environmental reference materials, food and occupational health, was certainly favoured by the fact that both the environmental research programme at Ispra, with ramifications into food analysis, the workplace environment and other priority areas, and the BCR programme developed in parallel. This joint effort is a reminder that progress in the protection of the environment, and in the development of the appropriate legal instruments, depends vitally on the quality of measurements, which in turn require matrix-true certified reference materials for their verification and validation. As soon as analytical problems arose, e.g. new classes of analytes and matrices, calling for method and data validation, new reference materials were requested by the analytical community. The Ispra reference laboratory could evaluate the possibilities of producing such a material, and test its properties, and BCR typically was able to react and take decisions rather quickly since both METRE and BCR were controlled by the same Advisory Committee of Programme Management.

The evolution of the BCR over the past 26 years is marked by many significant changes in both R&D programme content and formal naming. The first programmes — BCR-1 (1976–78) and BCR-2 (1979–82) — were dedicated to the support of industry, with a small but growing share of environmental CRMs. In 1979, applied metrology was added to the BCR's agenda in order to promote Europe's measurement infrastructure, refining also the certification procedures and consolidating its position in the global reference material market. BCR-3 (1983–87) already marked a sharp peak in environmental CRM production.

Although CRM production remained the cornerstone of the BCR programme, further activities were added. These aimed at building laboratory competence and qualifications in new fields of reference material production, or simply improving the performance of a selected group of laboratories up to the level needed for certification through collaborative studies. The first of these collaborative studies, undertaken in the early 1980s for the determination of PCBs in environmental matrices, was crowned in 1986 by the certification of seven PCB congeners in BCR 292 sewage sludge. Several other studies, such as PAHs and dioxins, followed, again catalysing progress in specific sectors of analytical sciences, and consequently in environmental analysis.

With the Framework Programme becoming established as the main instrument of Community-wide research, the BCR had to become part of it. Major objectives of the programme were to harmonise measurements across the Member States, to establish traceability to the primary SI units and to provide accurate measurements to support

European policies. With the development of the Single Market the exchange of goods and services required agreement on chemical, biological and physical measurements between trade partners, and research demands arose for a wide variety of sectors. Most importantly in the field of environmental analysis, the first CRMs for element speciation and fractionation for metal mobility assessment were issued.

Despite all changes, however, it is gratifying to note that the Certified Reference Materials produced by the European Commission's services are still called BCR CRMs after the early BCR Programme.

The history of the European Commission's efforts in the field of measurement quality improvement recalls another most important development: following the closure of the METRE Programme in 1984, it was decided to concentrate all future reference material activities at Geel in the premises of the CBMN, giving rise to the construction of what is today the world's most advanced reference material servicing laboratory. The Institute of Reference Materials and Measurements (IRMM), as it was re-named, has since 1994 had responsibility for the storage, sale and distribution of CRMs, as well as their replacement when sold out. To date, over 400 CRMs are on the sales list, covering a wide range of sectors, including biomedicine, the environment, food and animal feed, water and waste, pure chemicals and pharmaceutical products. A dozen or so new CRMs are added every year.

Europe is growing together and it is growing larger: fifteen countries, with very different histories and traditions, are trying to determine the rules of the game which will govern their future. Six candidate countries eagerly await their entry. All must meet countless environmental regulations and industrial quality requirements, which means measurements, improvement of data quality and hence more CRMs.

Already the demand for CRMs is growing at a rate of 20% per year, while existing producers cannot meet the requirements, due to budget cuts. We should remember that the cost of certifying reference materials, especially matrix reference materials, is high. As the procedures preferred by the BCR are stringent, users in fact pay only a small part of the real cost. Should we therefore lower our quality requirements in the production of CRMs or discourage their frequent use? Certainly not. But we could encourage a more appropriate use. Much too often, CRMs are employed in the construction of daily quality-control charts, distributed in interlaboratory exercises, improperly used as calibrants, etc. Certainly, there might be situations where CRMs have to be the basis of calibration since any other solution might be deleterious for the quality of the measurement results, but they should be the exception rather than the rule.

One solution to the problem may be to reduce the demand for CRMs by making available secondary RMs, designed and produced according to the quality standards established and implemented today for CRM production and traceable to CRMs. Such materials might find a use in all kinds of day-to-day quality control procedures and similar measures. This solution may lower the cost of production; however, this approach is not applicable to all situations (e.g. CRMs are still lacking in many instances and the traceability link of secondary RMs to CRMs is questionable).

To some extent such materials might be produced under the umbrella of the European Commission or some other state-supported organisation. However, the quality of the production will have to be demonstrated through the ISO 9001 certification of procedures,

and follow the appropriate ISO guides concerning the production of reference materials. Producers should also have the capability to support the customer who buys and uses the product.

While it is hardly possible accurately to assess the growing need for either CRMs or RMs, in the medium term we may assume that it will be very high, considering the increasing quest for total quality in all types of measurements and the needs arising from environmental problems within an enlarged EU at the beginning of the next century.

Considering the trends in the development of measurement instruments within the next ten years, we expect that CRM demand could grow by about 5% per year. Because their use is growing from a smaller base, the demand for non-certified reference materials, e.g. for proficiency testing in support of laboratory accreditation, could increase by about 10% per year. It is assumed that the growth rate for CRMs will remain constant at the 20-year horizon but that the use of non-certified RMs will grow at a rate of 15% per year. Within 30 years from now, one might expect that the increasing sophistication of measurement instruments will enable us (at least in some advanced fields of trace analysis) to get rid of the inherent difficulties of validation, and the use of matrix CRMs could hence decrease. While it is likely that the main demand will be for the continuous replacement of obsolete and sold-out materials with new types of materials to address the challenges, demand for non-certified reference materials linked to accreditation might slow down by about 5% per year.

Herbert Muntau

Abbreviations

AAS	Atomic absorption spectrometry
ADPCSV	Adsorptive differential pulse cathodic stripping voltammetry
AFS	Atomic fluorescence spectrometry
AOAC	Association of Official Analytical Chemists
BCIC	Bomb combustion followed by ion chromatography
BCR	Bureau Communautaire de Référence (European Commission)
CATH	Catharometric detection
CEN	European Committee for Standardisation
Cfp	Colony forming particles
CGC	Capillary gas chromatography
CRSTC	Combustion followed by chromatographic separation and catharometric detection
CRM	Certified reference material
CSCAT	Catharometry applied after combustion and chemical separation
CSV	Cathodic stripping voltammetry
CV	Coefficient of variation
CVAAS	Cold vapour atomic absorption spectrometry
CVAFS	Cold vapour atomic fluorescence spectrometry
DCP-AES	Direct current plasma atomic emission spectrometry
DMA	Dimethylarsinic acid
DPASV	Differential pulse anodic stripping voltammetry
DPCSV	Differential pulse cathodic stripping voltammetry
DPP	Differential pulse polarography
ECD	Electron capture detection
EDAT	Eschka digestion followed by argentometric titration
EDVT	Eschka digestion followed by Volhard titration
EDXRF	Energy-dispersive X-ray fluorescence
EPA	Environmental Protection Agency
Et	Ethylation
ETAAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FAES	Flame atomic emission spectrometry
FID	Flame ionisation detection
FPD	Flame photometric detection
FPSA	Flow potentiometric stripping analysis
FLUO	Spectrofluorimetry
FTIR	Fourier transform infrared spectroscopy

GRAV	Gravimetry
HDPE	High density polyethylene
HG	Hydride generation
HGAAS	Hydride generation atomic absorption spectrometry
HICP	Hydride generation inductively coupled plasma atomic spectrometry
HPLC	High performance liquid chromatography
HTAT	High temperature combustion followed by acidimetric titration
GC	Gas chromatography
GSCAT	Catharometry applied after combustion and GC separation
IAEA	International Atomic Energy Agency
IC	Ion chromatography
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ID-ICPMS	Isotope dilution ICP-MS
IDF	International Dairy Federation
IDMS	Isotope dilution mass spectrometry
ID-ICPMS	Isotope dilution ICPMS
IE	Ion exchange
INAA	Instrumental neutron activation analysis
IRMM	Institute for Reference Materials and Measurement
ISO	International Standardisation Organisation
KJEL	Kjeldahl-type determination: acid digestion followed by steam distillation and acidimetric detection
KFA	Kernforschungsanlage
LAES	Liquid anion exchange solution
MIBK	Methyl isobutyl ketone
MIP-AES	Microwave induced plasma atomic emission spectrometry
MS	Mass spectrometry
NIES	National Institute for Environmental Studies (Japan)
NIST	National Institute for Standards and Technology (USA)
NRCC	National Research Council of Canada
PAH	Polyaromatic hydrocarbon
PCB	Polychlorobiphenyls
PCDD	Polychloro dibenzo-dioxin
PCDF	Polychloro dibenzo-fluran
PAA	Proton activation analysis
PCSTC	Combustion, physico-chemical separation and catharometric detection
Pe	Pentylation
POT	Potentiometric titration
PTFE	Polytetrafluoroethylene
QFAAS	Quartz furnace AAS
RIVM	Rijksinstituut voor Volksgezondheid en Milieuhygiene
RFNAA	Activation with fast neutrons and radiochemical separation
RNAA	Neutron activation analysis with radiochemical separation
RPAA	Proton activation with radiochemical separation

RREX	Radioactive radiochemical extraction
SFE	Supercritical fluid extraction
SPEC	Visible light or UV spectrometry
SS-ZETAAS	Solid sampling ZETAAS
SWASV	Square wave anodic stripping voltammetry
TCPT	Tube combustion followed by argentometric titration
TIMS	Thermo-ionisation mass spectrometry
TI-IDMS	Thermo-ionisation isotope dilution mass spectrometry
TITR	Titrimetry
TVOL	Volhard titration
TXRF	Total reflection X-ray fluorescence
UV	UV irradiation
VOL	Volumetric measurement of gas
XRF	X-ray fluorescence spectrometry
ZETAAS	ETAAS with Zeeman background correction

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Chapter 1

General introduction

1.1. THE ANALYST AND HIS DUTY: SOCIO-ECONOMIC IMPACT OF ANALYTICAL ACTIVITIES

Chemical and biological measurement are performed daily by thousands of analysts around the world. Their objective is to study the properties of matter and to describe and quantify them. Chemical and biological measurements are produced in all fields of human technical or scientific activities. A major proportion of them are performed within industry. They serve as control tools for the quality of raw materials and of intermediate or final products. The verification of products may have a sole internal quality-control objective (conformity of production to present quality characteristics or customer demands etc.) and/or they may have regulatory purposes (e.g. product safety, conformity to standards etc.). They also play a key role in the monitoring of the production processes, in the control of the potential impact of production on the environment or on safety in the workplace, etc. Depending on the type of industry or of production, the economic impact of measurements can take up major parts of the industry's budget. They have a direct impact on cost, and an indirect effect through the decision linked to the measurement results, e.g. conformity or not of the products. Weindlmaier reported in a recent communication that measurements account for 5 to 15% of the total processing cost of industry and 4 to 8% of sales-related costs [1]. For the dairy industry, he estimated that between 1980 and 1994 the cost of analytical measurements increased from €1.35 per ton of processed milk to €2.85 per ton. Indirect impact on company income due to inappropriate conclusions drawn from improper measurements is not quantifiable precisely. In addition, secondary induced effects such as alteration of the image of a company due to improper or unsafe marketing of goods are difficult to estimate.

Besides industrial production-related measurements, modern society requires measurements and testing of diverse technical services. From industrial servicing, e.g. after-sales service, technical control of cars, aeroplanes etc., to environmental monitoring, biomedical measurements, food control etc., nowadays nearly everything is measured or tested. As early as 1977, Uriano and Gravatt [2] revealed figures for the impact of chemical measurements on the US Gross National Product that approached several billion US dollars per year in several fields of the economy (e.g. biomedical, agricultural). Hertz estimated, in 1988, that 0.5% to 8% of that GNP is directly affected by the quality of chemical measurements and that every day in the USA about 250 million measurements are performed at an annual cost of \$50 billion [3]. One in ten of the measurements performed has to be repeated because of mishandling: \$5 billion annually. Havelaar et al. give similar figures for microbiology measurements [4]. At the beginning of the

1990s about 200,000 microbiological measurements of drinking water and approximately one million for food control were performed by the Dutch Inspection Services every year in the Netherlands. Similar figures can be expected from all European Union countries, which leads to several million microbiological measurements per year just for regulatory control purposes. The European Commission [5] estimated that, in 1990 in the EU, about three million microbiological measurements were performed to monitor bathing water. If one counts an average estimate of €10 per analysis (without sampling costs), about €350 million are spent every year for microbiological regulatory control measurements. Figures reported by Havelaar et al. [4] reveal that, depending on the difficulty of the measurements, between 10 and 50% of the measurements lead to false-negative results (wrong results with potential risk-related effects). Several years of interlaboratory projects conducted by the European Commission under the BCR and other measurement and testing programmes have confirmed such quality data. All these figures do not include the economic or social induced effects of measurements, which are particularly important when wrong measurements are performed. If such effects cannot be estimated in terms of budget, they may be estimated from the resulting effects, e.g. epidemics due to food poisoning, displacement of population due to environmental pollution, restrictions in the workplace or closing of factories, interruptions of water supply, withdrawal of batches of manufactured products etc. This gives already an indication of the challenge for analytical chemists and microbiologists.

As such, chemical or biological analyses do not differ in principle from physical measurements. The analytical approach is similar: to decompose a problem into understandable, controllable and finally measurable quantities. The metrological difficulty for chemists and biologists lies more in the diversity and the complexity of the measurand, and the diversity and complexity of the samples. Where in physical measurements the quantity to be measured is often directly accessible, in chemistry and biology difficult handling stages of the sample itself are required. These stages may change from sample to sample or with the quantity to be measured. Due to this complexity of measurements, analytical chemists and biologists have difficulties in achieving and maintaining comparability of results. At the beginning of the 21st century, in a rapidly changing world, analysts are increasingly asked to demonstrate evidence of facts, situations or the value of goods or services. Many relations in modern society have become technical and consequently decisions rely more and more on quantified facts. This applies to trade, industry, the environment and health protection, as well as safety, but also to justice (criminology) or even the understanding and the protection of our cultural heritage. Consumers, lawyers, regulators, politicians, lobbies of all kinds, pressure groups and all those who request analytical services are not satisfied simply with data but want answers to their questions. In addition they ask for certainties in the quality of these answers. Quality answers rely on quality data, and such data can only be produced and documented if the laboratory works within a proper quality system. Data are only accepted as being reliable if the quality system of the laboratory is recognised by the scientific, analytical, economic and legal systems. Several systems exist and benefit from a mutual recognition of those who adhere to them. They are described in section 1.4.

1.2. THE NECESSITY TO DELIVER GOOD ANSWERS

As already stated above, modern society requires answers from the analyst, i.e. not only simple data. In practice this means that the responsibility of the analyst has increased. When he is obliged to give answers — e.g. did the mercury contamination of this river increase, decrease or is it stable? — he cannot just give the concentration in mercury from samples whose origin is unknown to him. He must therefore involve himself in the sampling process. It also means that he has to keep a record of previous measurements (or retrieve it from other sources), and know the variability of his measurement as well as of the sampling technique (not to speak of the variability in the samples). This also requires his involvement at all stages of the planning of the study itself so that he can exercise his judgement and experience and finally engage his responsibility. When he delivers his answer this is done in a manner which is scientifically acceptable, understandable, and useful to his customer. If the customer is not satisfied with the answer, the analyst records complaints, analyses the reason and must redo the work. Such working procedures and behaviour can be called a ‘total quality’ approach.

In practice, what does this imply? Without going into detail, it must be stated that such a way of working is only possible if the laboratory has foreseen — *a priori* action — that repetition of the task can be undertaken and that this is the way customers should be handled. Quality work can only be produced in an organised system: a quality system. It involves all aspects of the laboratory that affect the production of results: people (analysts, managers, administrators), infrastructure (buildings, equipment), supply (chemicals, reference materials etc.) and control of third parties (maintenance companies, security auditors etc.). Everything must be up to date: goods and people.

1.3. THE NECESSITY TO PRODUCE RELIABLE DATA

To deliver reliable answers to the customer, the analyst must be involved from the start, when the problem to be solved is posed. To be a reliable partner in such an instance he must be able to demonstrate that his laboratory benefits from an adequate infrastructure and standard of quality management, i.e. that he has the basic tools to deliver the proper analytical data that will be the basis of his final answer. Proper results follow the classical procedure shown in Figure 1.1. When a problem is raised, its study passes from an evaluation of needs to the planning of a strategy. The analytical work itself follows, and finally the exploitation and proper presentation of the results completes the circle. When solving the problem needs several days, months or years — which is an increasingly common situation when environmental studies are tackled — or it foresees the study of large areas or large series of samples to monitor production, then the quality circle of Figure 1.1 reveals its importance. Preliminary investigations after the first measurements and exploitation of results will evaluate if the entire procedure was adequate. If not a new circle with an additional input of knowledge for the planning is set up, starting a type of quality spiral. Reconsideration of the circle can be due to

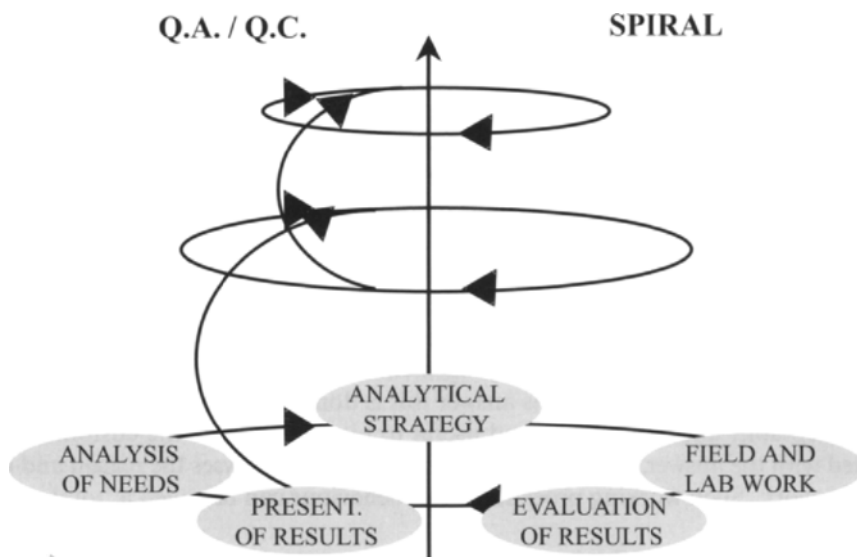


Fig. 1.1. The various steps of the quality assurance and quality control of the analytical laboratory can be presented on a circle, from the problem definition to the answer to the customer. When properly organised the last step leads to useful answers to the question posed by the customer. Alternatively, when the answers are not fully adequate, the analyst and the customer redefine another approach based on the results of the first circle. Due to the experience of the first investigation, this second circle has an increased chance of solving the problem. It is the start of a quality spiral (adapted from ref. [10]).

the type of parameter(s) measured, or may come from a non-adaptive way of expressing the results (e.g. dry mass instead of fat content), or the improper precision of the applied method. It can also be induced by insufficient information obtained from the collected samples and therefore more samples or more adequate ones must be measured. Depending on the quality of the first analysis of needs, the reconsideration of the analytical approach may induce fully different economic figures for the customer.

Many factors influence the set up of the quality spiral: primarily structural items like equipment, infrastructure or personnel. Management, external constraints of commercial or regulatory origin, e.g. accreditation, also affect the laboratory work. They are highlighted in Figure 1.2.

Many tools are in the hands of the analyst and management to adapt the laboratory to his task. Figure 1.3 shows that they are of various types. They have to be included in the entire management of the laboratory, e.g. training of staff and motivation must be planned and built in the lifestyle and the lifetime of the laboratory. Quality always pays or as W. Edwards Deming preached: 'cheaper is not always better, better is always cheaper'. It may appear to be expensive and time consuming, but it is the only way to cope with unexpected situations, e.g. absence of staff, sudden unexpected workload etc. It is out of the scope of this book to discuss all infrastructure and management aspects necessary to set up a quality system within a laboratory.

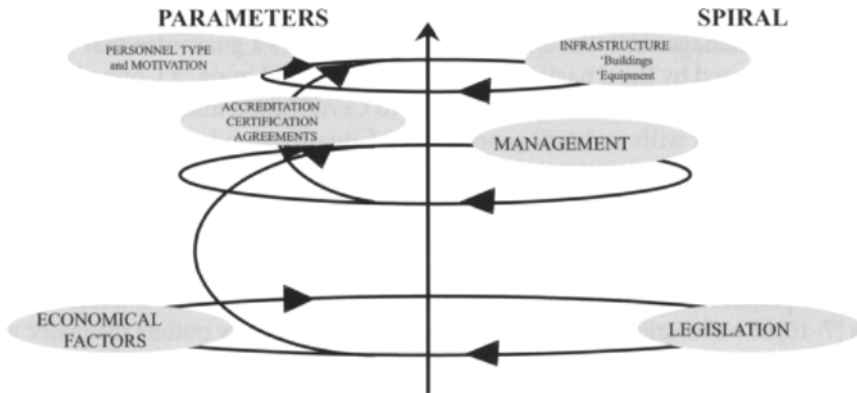


Fig. 1.2. Quality assurance and quality control are strongly influenced by internal structural and external regulatory parameters. When planning the work, the laboratory must take them into account (adapted from ref. [10]).

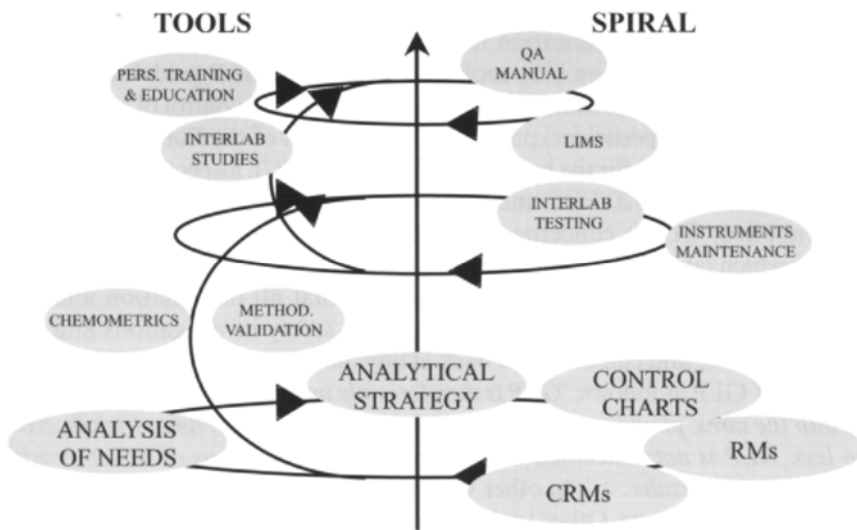


Fig. 1.3. The quality spiral described in Fig. 1.1 and its implementation depend on the tools available in the laboratory. These tools concern laboratory structure, personnel, management, external and internal quality assessment measures. The proper use of the available tools will lead to the development of the quality objectives (adapted from ref. [10]).

1.4. THE REGULATORY BACKGROUND OF QUALITY

The laboratory management is not alone in preparing and setting up a quality system. Following the examples of others who have gone the same route will help. In addition, for several years now, regulatory and standardisation bodies have produced a series of

quality management standards which give the framework for such an activity. Following them gives the management, the analyst and the customers a guarantee that the quality system is recognised by third parties. Within the European Union, a Council resolution of 21 December 1989 on the Global Approach to Certification and Testing [6] provided the Internal Market with an homogeneous set of quality standards based on the ISO 9000 and EN 45000 series of standards. Besides these two series, other quality standards are in force in some particular branches of industry, e.g. GLP in the pharmaceutical industry, or a more extended view, e.g. the ISO 14000 series including environmental aspects into ISO 9000.

How to prepare for certification or accreditation is a matter which is treated in other books [7–10] or publications [11]. We will just indicate and briefly outline the major world standards that tackle with quality systems and in particular those concerning analysts.

1.4.1. Good Laboratory Practice (GLP)

The GLP system was first developed in 1978 by the US Food and Drug Administration (FDA). In 1983 the US Environmental Protection Agency (EPA) issued similar regulations for the production of agricultural and industrial toxic chemicals. Following pioneer work by the WHO, the Organisation for Economic Co-operation and Development (OECD) in its C81/30 decision [12], took over in 1981 the GLP guidelines which then became the rule in all OECD member countries for the quality control of pharmaceutical and toxic chemical (e.g. pesticides) producers. The Council of the European Community adopted several Directives for the harmonisation (87/18/EEC), inspection and verification of GLP regulations (88/320/EEC and 90/18/EEC).

Where applied, GLP is concerned with the organisational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported [12]. In fact, GLP intends to guarantee that all information and actions undertaken in the course of product development, safety investigations and toxicity testing are traceable and can be found back at any moment. Merz et al. [13] summarise the objective of GLP as follows: *'GLP is therefore only intended to provide a comprehensive insight into the aims, planning, performance, evaluation and reporting of trials. No more and no less. GLP is not concerned with the requirements or aims of a test or with the interpretation of its results. . . .'* In other words, following GLP rules does not guarantee the accuracy of test results. Other tools, such as the use of CRMs and participation in proficiency testing studies, allow the demonstration of accuracy. GLP as well as any other quality systems only improves the quality of the working environment and the way laboratories function. Doing so, they recommend measures to achieve accuracy.

1.4.2. Accreditation systems EN 45000 and ISO 17025

GLP guidelines have been set up for toxicological investigations of newly developed chemicals or pharmaceuticals. Gradually, more and more scientific areas and types of industries have been involved in GLP, especially for safety aspects of new products. General testing laboratories are usually not concerned and the GLP rules are mostly not applied to their work. For such laboratories or for other type of industries, the

authorities have set other quality systems, some of which have been in existence for several years. Depending on the field of activity, e.g. food, environment, biomedical analysis, the control systems took the form of administrative inspections, proficiency testing or both simultaneously. In several countries, the control systems for testing laboratories are based on the ISO/IEC Guide 25 issued in 1978 and last revised in 1997 [14]. Within the European Union, the resolution 90/C/10/01 of the Council of Ministers of 21 December 1989 on '*a global approach to conformity assessment*' [12], adopted guiding principles mentioned in the European series of norms EN 45000 [15] for testing laboratories. In fact, the testing laboratory accreditation system involves six standards:

- EN 45001: general criteria for the operation of testing laboratories;
- EN 45002: general criteria for the assessment of testing laboratories;
- EN 45003: general criteria for laboratory accreditation bodies;
- EN 45011: general criteria for certification bodies operating product certification;
- EN 45012: general criteria for certification bodies operating Quality System certification;
- EN 45013: general criteria for certification bodies operating certification of personnel;
- EN 45014: general criteria for suppliers' declaration of conformity.

The Council resolution promotes a mutual recognition principle between the accreditation systems developed in the various member states. The EN 45001 norm lists and recommends a number of quality assurance and quality control items to be operated by testing laboratories. These include recommendations on the management, infrastructure and competence of personnel, equipment and working procedures and, in particular, detailed aspects with regards to calibration and use of reference materials, and participation in proficiency testing schemes. The EN 45002 norm clearly states that the accreditation of a laboratory cannot be granted or maintained exclusively on the basis of the results obtained by a laboratory in proficiency testing, obliging the accreditation body to perform audits. Several European countries not belonging to the European Union, e.g. those who are part of the European Free Trade Agreement (EFTA), have adopted the same system. All accreditation bodies of Western Europe are co-operating within the European Accreditation of laboratories (EA) for a common implementation and development of their systems. Outside Europe, similar accreditation systems are operated under the ISO Guide 25 principles e.g. NATA in Australia, A2LA in USA. On a global level the accreditation bodies are co-operating within the International Laboratory Accreditation Cooperation (ILAC). This ISO Guide 25 is in course of full revision and should become the ISO 17025 standard, which would then replace EN 45000. Several international scientific societies e.g. IUPAC, AOAC, also associate their efforts to develop common approaches, a common vocabulary and to set up common guidelines or common means and tools for the improvement of the quality in testing laboratories.

1.4.3. ISO 9000 series

GLP requirements have the objective to ensure public safety for chemical products. The ISO 9000 series of standards [17] is intended to ensure quality for manufacturing and services companies. In 1987, the European Standards Institution

CEN/CENELEC/ETSI — took over and improved the ISO 9000 standards as the EN 29000 series that were included into the Council resolution 90/C/10/01 of 21 December 1989. Since then the ISO 9000 series has been revised and replaces fully the EN 29000. The ISO 9000 series of norms include five standards:

- ISO 9000: general guidelines, principles and definitions;
- ISO 9001: quality system specifications and requirements for design of production and service;
- ISO 9002: quality system specifications and requirements for production and installation;
- ISO 9003: quality system specifications and requirements for final inspection and testing;
- ISO 9004: guidance on quality management.

In ISO 9001, 9002 and 9003, measurements and quality requirements for measurements or tests are required. Therefore, analytical chemists employed in industries or services pursuing ISO 9000 certification will see their analytical activity audited and controlled.

1.4.4. Accreditation/certification of RM and CRM producers

Reference materials and certified reference materials are essential tools for the assessment of the reliability and comparability of analytical results. Therefore, the quality of the RMs and CRMs, and the scientific and technical ability of the producers should be assessed. The number of producers, especially of RMs, has increased over the last years and quality of the produced materials was not always demonstrated. In order to help producers to perform their task in the most reliable manner and to allow customers to estimate the competence of RM or CRM producers, ISO has started a project on the quality requirements for the production of RMs and CRMs. ISO Guide 34 giving the interpretation of ISO 9000 and ISO Guide 25 has been adopted in 1996 [18]. This Guide covers various requirements and items:

- *Organisational requirements*: management, quality policy and system, staffing and training, contracts and collaboration, storage and long term monitoring of RM and CRM stability, recording and reporting, after-sales service;
- *Production control*: planning, preparation of materials, traceability and calibration, measurement equipment and methods, assessment of homogeneity and stability, data treatment and certification.

The technical requirements are those set up in the ISO Guides 31 [19] and ISO Guide 35 [20]. These two Guides will be discussed extensively in the following chapters of this book.

1.4.5. Written standards and standardisation bodies

Written standards (norms) represent a way of defining and promoting requirements of quality of products and for improving comparability of analytical results. Written standards are also a first step for the introduction into regulatory systems of these minimal requirements of quality and comparability. Often, regulations at the legal or commercial level refer to standards for the implementation of the regulation. In the

field of analytical chemistry, written standards have shown their importance for many applications, such as sampling strategies and techniques, the definition and the measurement of certain global parameters, e.g. total organic carbon, total organic halogens, or fractions of substances such as extractable, leachable and bioavailable fractions of compounds.

In other fields, analysts often regret the existence of written standards especially when they are bound to regulations. This situation is encountered for standardised analytical methods that are outdated but still mandatory as the legislation has not been revised. In situations where the state of the art has improved the analyst may be obliged to use old-fashioned methods just for legal reasons. Such situations are often encountered in the pharmaceutical field, in food control or environmental monitoring. The application of a standardised method is no guarantee that errors will not affect the analytical work. In fact, often large disagreements have been noticed in BCR projects between laboratories applying the same standard [21]. This may be due to systematic errors, improper execution of the method by operators or to the fact that the wording of the standard is ambiguous or does not cover everything. Standardisation bodies have recognised the difficulty of applying standards and the necessity to allow progress in analytical sciences to impact standardised methods. Therefore, in recent written standards more general analytical approaches are used, giving general principles for methods with performance characteristics to be achieved. Such standards may also recommend demonstrating that the requested performance is achieved, i.e. upon the analysis of CRMs. An example is given by the European standards on the incineration of dangerous waste, for the emission of dioxins and furans [22].

Standardisation bodies exist at national, regional and international level. They may depend upon public authorities entirely or partly or they may belong to professional or commercial organisations. For European countries organised in the European Union and the European Free Trade Association, CEN/CENELEC/ETSI produce European standards which are gradually replacing national standards. At international level more than one hundred countries are collaborating in ISO. ISO and CEN have passed an agreement in 1991 in Vienna to avoid overlap of tasks. Following this agreement, CEN takes over some written standards developed in ISO when they fulfil the needs of CEN. ISO and CEN cover large fields of activities. Hundreds of technical committees (TCs) each support the work of several working groups. Professional organisations are also producing written standards that may also be adopted by ISO or CEN afterwards. A typical example is given by the International Dairy Federation. IDF produces standards for milk and dairy products, which are regularly adopted by ISO.

1.4.6. Conclusions

Within the last decade, quality management of laboratories has become globally accepted and organised. Laboratory managers have at their disposal a series of standards produced by ISO or CEN, which guarantee that their organisational aspects are recognised abroad. The main standards have been described above; others have been

recently developed and are less known, e.g. the ISO 14000 series, which extends the ISO 9000 series towards environmental impact [23].

These standards do not guarantee that analytical results will always be perfect. Errors still can be made. Therefore, within these quality standards a number of activities and actions must be taken to deliver reliable results. These aspects and in particular those linked to reference materials and interlaboratory studies will be discussed further in this book.

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Chapter 2

Quality in chemical and biological analysis

2.1. TOOLS TO ACHIEVE QUALITY

Before starting speaking about tools, it must be clarified that the words used are understood and used with their proper meaning. Having recalled the most recent versions of definition of terms, it will be necessary to investigate which tools analysts need to achieve their objectives. The ultimate objective is, as said before, to deliver reliable and adapted answers to customers. We will then examine how reference materials and certified reference materials can help to verify the reliability of analytical procedures. We will also see how interlaboratory studies can benefit analytical quality work.

2.1.1. Terms and definitions used in analytical sciences

2.1.1.1. Basic definitions

A certain number of basic principles and rules govern all analytical sciences. In particular, performance criteria and their definitions are (or at least should be) the same for all analysts. These performance criteria govern the validation process. Nevertheless, in some analytical disciplines differences may appear due to the measurand concerned. In particular, when approaching the metrology of living organisms (e.g. bacteria, viruses, parasites, cells) or biological activity related parameters (e.g. vaccines activity) definitions may suffer compromises. These definitions are taken from the *International Vocabulary of Basic and General Terms in Metrology* (BIPM) [1], *ISO 3534 — Statistics — Vocabulary and Symbols* [2], and *Vocabulary of Legal Metrology* (OIML) [3].

In the field of biological metrology some nuances have to be brought into the definitions. Havelaar and co-workers have tried to transpose these definitions into terms that appeal to microbiologists [4,5]. These definitions can also be found in the guidelines for microbiologists edited by Lightfoot and Maier [6].

Random error: a component of the error of a measurement which, in the course of a number of measurements of the same measurand, varies in an unpredictable way [1].

Systematic error: a component of the error of a measurement which, in the course of a number of measurements of the same measurand, remains constant or varies in a predictable way [1].

Bias: the difference between the expectation of the test result and an accepted reference value. Bias is a systematic error as contrasted to random error [2].

Precision: the closeness of agreement between independent results obtained under prescribed conditions [2]. Precision covers repeatability and reproducibility [2]:

Repeatability: precision under repeatability conditions, i.e. conditions where independent

test results are obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

Repeatability limit r: the value less than or equal to which the absolute difference between two single test results under repeatability conditions is expected to be with a probability of 95%.

Reproducibility: precision under reproducibility conditions i.e. conditions where test results are obtained with the same method on identical material in different laboratories, by different operators using the different equipment.

Reproducibility limit R: the value less than or equal to which the absolute difference between two single test results under reproducibility conditions is expected to be with a probability of 95%.

Trueness: the closeness of agreement between the average value obtained from large series of test results and an accepted reference value. The measure of trueness is usually expressed in terms of bias [2].

True value (of a quantity): a value, which characterises a quantity perfectly defined, under the conditions which exist when that quantity is considered [1]. The vocabulary of legal metrology gives a definition of the true value which makes a direct reference to the measurement: a value which would be obtained by measurement, if the quantity could be completely defined and if all measurement imperfections could be eliminated [3].

Accuracy: (trueness + precision) the closeness of agreement between a test result and the accepted reference value [2].

Uncertainty (of measurement): parameter, associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand [1].

Robustness, ruggedness: the ability of a method to be relatively insensitive to minor changes in the procedure, to quality of reagents or to the environment [7]. These are terms used in correlation with methods applied for the measurement of numerous samples, not always fully similar in composition, over long periods of time. It expresses the flexibility of the method or the ability of the method to remain unaffected by slight changes of conditions. Such changes may be: temperature variations and effect on electronics, ageing of chromatographic columns, variations in the water content of samples, slight differences in matrix composition, replacement of parts of an equipment, other operator, variation in the environment temperature, humidity, light etc.) and many others.

2.1.1.2. Trivial terms

The selection of an analytical method may also be influenced by external regulatory requirements or by special requests of the customer. The following terms often appear in the analytical jargon and were compiled by Garfield [8]; the classification of methods relies on purpose of the method or the administrative background.

Official method: a method required by law or by a regulation issued by an official agency (e.g. EPA, FDA, European Directives, etc.).

Reference or standard consensus methods: methods developed by organisations that use interlaboratory studies to validate them (ISO, CEN, AOAC, DIN, BSI, AFNOR etc.). Their development leads to a known and stated precision or accuracy.

Modified method: reference or standard method which has been modified to simplify or adapt it to the actual state of the art or to other types of samples.

Rapid methods: methods for the rapid determination of large number of samples. Such methods are of increasing interest in microbiology measurements. Hours or even days can be saved by reducing analytical time; it increases throughput and consequently reduces cost.

Routine methods: methods used on a routine basis in daily practice. They may be official or standard methods.

Automated methods: methods using automated equipment.

Destructive methods: the determination of the amount of a substance in a material usually leads to the destruction of the test sample. This is realised by pretreatment techniques such as acid digestion, fusion, extractions etc. to bring the test sample into a liquid or other simple form compatible with the final determination technique or instrument. Nearly all modern methods of measurements, e.g. spectrometry, require sample pretreatment procedures.

Nondestructive methods: such instrumental methods in fact only require simple pretreatment which does not require the extemporaneous physical destruction of the test sample. In fact, some of so-called nondestructive techniques do not leave the sample unaffected. Instrumental Neutron Activation Analysis affects the sample in so far that the elements are radioactively transformed. The sample after analysis becomes radioactive and cannot be considered as unaffected! Fully nondestructive techniques are limited to a few number of techniques applied in certain situations; they are particularly rare for the quantitative analysis of solid materials: ^1H NMR, NIR, Raman, XRF and related techniques, etc.

2.1.1.2. Hierarchy of methods

With regard to the hierarchy of methods, primary methods or definitive methods frequently represent the highest level of reliability. Such concepts, coming from fundamental physical metrology, may find their interest in particular within discussions on traceability concepts (see section 2.1.3). In chapter 5, on the certification of RMs, these aspects are discussed in some more detail (section 5.1.4.1).

Primary method: a primary method of measurement is a method having the highest metrological qualities, the operation of which can be completely described and understood, for which a complete uncertainty statement can be written down in terms of S.I. units, and whose results are, therefore, accepted without reference to a standard of the quantity being measured [9]. The Comité Consultatif sur la Quantité de Matière (CCQM) of the Bureau International des Poids et Mesures (BIPM) recognised as primary methods: isotope dilution mass spectrometry (IDMS), coulometry, gravimetry for gas mixtures and inorganic substances, titrimetry, determination of freezing-point depression, differential scanning coulometry.

Definitive method: method with a high scientific status applied in a laboratory of high proven quality [10]. In brief it means that only negligible systematic errors may remain compared to the precision and trueness required for the final result. Isotope Dilution Mass Spectrometry (IDMS), in its thermo-ionisation mode, for the determination

of traces of multi-isotopic elements, may be considered as a definitive method when applied in certain laboratories (e.g. laboratories determining the composition of fuels for nuclear fission). Associated to ICP ionisation the IDMS principle only starts to be used. In organic trace analysis, isotope dilution is not a definitive method.

2.1.1.3. Calibration linked classification of methods

The ISO Guide 32 on calibration of chemical analysis and the use of certified reference materials [11] classifies chemical methods into three categories with regards to the calibration procedure.

Calibration: the set of operations which establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure, and the corresponding known values of a measurand [1].

Type I or calculable or absolute method: a method that produces the anticipated result by performing a calculation defined on the basis of the laws governing the physical and chemical parameters involved, using measurements taken during the analysis, such as: weight of the test sample, volume of titration reagent, weight of precipitate, volume of titration product generated [11]. The analyst has to identify every quantity whose measurement is necessary to calculate the end result and to establish the uncertainty of this quantity. Several examples of such types of determinations are known in chemical analysis: e.g. titrimetry, coulometry, gravimetry. The calculable method cover in large parts the 'primary methods' defined by Kaarls and Quin [9]. It should be noted that there are no primary methods available for measurements of organic or organo-metallic and certainly not for biological substances or entities.

Type II or relative method: a method which compares the sample to be analysed with a set of calibration samples of known content, using a detection system for which the response (ideally linear) is recognised in the relevant working area (without necessarily being calculable by theory). The value of the sample is determined by interpolation of the sample signal and with respect to the response curve of the calibration samples [11]. Differences between sample and calibration sets have no effect or are negligible compared to the uncertainty on the signal. This implies a pretreatment of the sample, matrix matching of the calibration sets, elimination of interference etc. Modern spectrometric systems belong to these types of methods. Such methods cover nearly all organic, biochemical or organo-metallic trace analysis, and a vast majority of inorganic measurements.

Type III or comparative method: a method where the sample to be analysed is compared to a set of calibration samples, using a detection system which has to be recognised to be sensitive not only to the content of elements or molecules to be analysed but also to differences of matrix [11]. Ignoring any difference in the matrix will lead to errors. Calibration of such methods requires (Certified) Reference Materials ((C)RMs) with a known matrix composition similar to the matrix of the sample. Such methods are rapid and are often used in monitoring of manufacturing processes (e.g. WDXRF in the production of metals, alloys, coal, cement, powdered oxides, etc.) or for the determination of basic parameters (e.g. viscosity, particle size distribution etc.).

The three categories of methods differ by the way the content of the substance is established. In other words, the way the signal generated by the substance present in the sample is linked to the signal of the substance in the calibration material and consequently to its concentration. This link can be made directly to an amount of substance of established purity and stoichiometry in the case of calculable and relative methods (when all steps of the procedure are well established) or through a Reference Material of known matrix composition certified for the concentration of the substance in the case of comparative methods. This link, if established through an unbroken chain of activities to appropriate measurement standards, is called *traceability*.

Traceability: the ability to trace the history, application or location of an item or activity, or similar items or activities, by means of recorded identification [10].

The primary objective of the validation of the analytical method (see section 2.1.3.) is to establish the traceability of the result to the recognised reference (pure substance, characterised organism or CRM) in absolute terms or through a well established method. In practice traceability implies the existence of means to recover and verify the information.

For measurement sciences traceability can be expressed into more practical terms. The VIM [1] defines *traceability* as: the property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.

In analytical chemical terms this means: all steps of the analytical procedure should be performed and recorded in such a way that all essential information is available and no wrong information is introduced. In other words, the results of the determination and not only the final measurement should be linked through an unbroken chain of comparisons. This link must be demonstrated. Depending on the type of analytical method applied and the property of interest to be determined, various steps may be necessary to demonstrate the link between the end signal recorded from the detector and the reference to which it is linked.

For microbiology measurements, calibration is not performed as such. In fact the analyst often counts colonies (bacteria) or plaques (holes) in cultures (viruses, phages) or 'positive tubes' (most probable number techniques) which are supposed to be issued from one single biological particle. The growth of the 'particle' depends on the medium in which this growth is done.

2.1.1.4. Performance criteria of methods

In principle, the quality of an analytical method can be expressed through two groups of basic and secondary characteristics or figures of merit, which must be quantified by the user of the method before using it to deliver results. The determination of these quantified figures is achieved in the *method validation process* (see section 2.3).

basic characteristics:

- precision (repeatability and reproducibility)
- trueness
- sensitivity (limit of detection and determination)

secondary characteristics:

- specificity or selectivity
- range of linear response
- robustness and ruggedness

The specificity or the selectivity guarantees that the method is really measuring the substance of interest. Interferences of all types affect the specificity of several methods e.g. chromatographic separations with non-specific detectors such as FID or ECD, etc.

Specificity is the basic parameter for microbiology measurements and should for this field of metrology be placed first of the basic characteristics. Nearly all the specificity of the final counting relies on the selectivity of the culture medium and growth conditions.

Sensitivity is mainly a limiting factor in trace analysis. It is evident that the method applied should be sensitive enough so that the concentration of substance to be determined is accessible (limit of detection and determination) or the colonies of microbes countable. It must also deliver a measurable difference for a small change in content. When the sensitivity of the method of final detection is a limiting factor the analyst may have several possibilities which all will influence the selection, optimisation and validation of the other steps of the procedure. He may:

- change the method of detection (of course only when available in the laboratory);
- increase the sample intake and adapt the pretreatment steps;
- concentrate the substance or organism at a certain stage of the procedure.

The determination of the property of interest in a simple solution will indicate to the analyst his working range in terms of sensitivity of the signal. The degree of trueness and precision are properties quantified in the validation process. Robustness or ruggedness concerns the ability of the method to remain unaffected by environmental changes (analyst, time, fluctuations in supplies, etc.). All six characteristics have to be established and quantified in the validation of the method (section 2.3).

2.2. TOOLS FOR QUALITY ASSURANCE AND QUALITY CONTROL OF BIOLOGICAL AND CHEMICAL MEASUREMENTS

Analytical quality control is a part of the total quality assurance programme of a laboratory. It should guarantee that all work is performed according to strict pre-established rules [12]. The quality control techniques are applied to verify that these pre-set requirements are met. Analytical quality control is usually set up within the framework of recognised standards e.g. ISO 9001 or 9002 [13] or EN 45001 [14], ISO 25 [15]. Others exist, in particular those applied in industry (production and services) e.g. GLP [16], or ISO 14000 which is mainly the ISO 9000 standard including environmental impact concerns [17]. All these standards include strong management aspects as they are the guarantee of proper organisation of tasks. Many other approaches to quality in analytical work could be developed. The advantage of the above mentioned standards lies in their international recognition by trade partners and justice departments of many countries.

In practice, quality concerns everybody in the laboratory from operators to top

management. Their specific responsibilities in analytical QC can be differentiated into three layers of control activities:

- first line checks: mainly operator responsibility
- second line checks: responsibility of the non-operational staff
- third line checks: under top management hands

The three check levels also concern different comparability or reliability levels. The operator is responsible for the delivery of reliable data at the bench level applying his analytical judgement within pre-set guidelines. The second level checks assure long term and between operator comparability of methods and results (e.g. control charts). The third level of control assures comparability to the external world (e.g. interlaboratory studies). A detailed discussion of these aspects for microbiology testing has been given by Havelaar et al. [4] and will be briefly discussed in section 2.3.3. General principles for chemistry will be recalled in the following section.

2.2.1. First line checks

They are in the hands of the operator or analyst as a means of self control within pre-set criteria and behaviour rules. They should be done under the supervision of a superior or senior analyst. They are applied before, during and after the analysis itself.

Before the analysis: Before any result is delivered, it has to be assured that the samples to be analysed are received properly in the laboratory (labelling, storage, registration etc.). Before the sample is touched the operator must have validated all equipment and analytical methods. The validation aspects are dealt with in section 2.3.

During the analysis: All information in particular sources of failures of critical control points are registered. This might lead to the decision to repeat an analysis already at this stage.

After the analysis: In addition to the analysis of the sample itself parallel actions may be taken to additionally verify the outcome of the work e.g. running solvent or procedure blanks, analysing duplicate samples or spiked samples etc.

Finally, control charts should be used to verify that the method remains under control after the validation. Their principle and use is described within section 2.4. They imply that proper reference materials are available.

2.2.2. Second line checks

They are organised by the laboratory management independently from the operators in order to assure comparability between analysts or instruments. They can be conducted by introducing into the sample throughput a duplicate sample or a reference material or even a certified reference material (if the latter cannot be recognised by the operator). These intra-laboratory studies are particularly important to verify that: quality standards are maintained, training periods of staff had successful outcomes, drastic increase of sample throughput does not affect quality of various operators, or for motivation of staff. Unknown check samples should not be identified and should not be introduced into the sample sets at regular time intervals. Comparison of control charts of various

operators performing similar measurements may also allow evaluating performance of staff and methods in time (ruggedness of methods).

2.2.3. Third line checks

The real quality of analytical data is achieved when the results are comparable to those of other laboratories or better when accuracy is reached and demonstrated. The top management of the laboratory must favour third line checks and give the responsibility of their organisation to the quality manager. External audits and comparisons with other laboratories must be part of the laboratory quality assurance programme. It is the only way of maintaining recognition by the analytical scientific community and consequently by the customers. They are strongly recommended by the accreditation bodies. Unfortunately, proper proficiency tests do not exist for all measurement fields (in particular for environmental monitoring analysis) or everywhere in the world. The use of certified reference materials is another way of linking laboratory results with the analytical community. The use of CRMs is under the laboratories' own responsibility and not dependent upon the existence of proficiency tests. Third line checks are dealt with in the chapters on interlaboratory studies (chapter 12) and the use of CRMs (chapter 3).

2.3. VALIDATION OF METHODS

When the analyst has properly defined the analytical problem to be solved (see Chapter 1), he has to select an adapted analytical procedure. The selection and the development of the procedure will be based on the investigation of the scientific literature and on the experience of the analyst and of colleagues. The general equipment of the laboratory and the scope of its activity will also influence the choice of method and the selected approach. Several books deal with the most important methods applied by analytical chemists. Examples of their application are given and the reader should refer to them. A basic book, mainly foreseen for students in analytical chemistry, but also very valuable for senior analysts, has been published recently by Kellner et al. [18].

All analytical methods are composed of a succession of actions:

- sampling, storage and preservation of a representative test sample of the material;
- pretreatment of a portion of the test sample for the quantification;
- calibration of the instrument and signal;
- final determination of the test sample;
- calculations and presentation of results.

The selection of the analytical method is usually done on the basis of the already available instrumental possibilities of the laboratory and the experience of the staff. The analytical procedure consists in the transformation of the test sample in order to convert the analyte into a physical or chemical form, which allows determining accurately the amount of a substance present in this test sample.

Selecting and developing a method consists in the choice of individual steps, to make them compatible and to develop tools to verify that the individual steps and the entire

procedure lead to reliable results. Reliability of analytical data means that they are precise and true. Precision is achieved when random errors are minimised. Trueness is reached when systematic errors are eliminated.

Fortunately, the analyst starts usually from an already advanced stage of knowledge due to the impressive literature available. Many standardised methods exist which have been published and updated by the standardisation bodies (e.g. ISO, CEN, professional bodies such as AOAC, IDF etc.). Many of these methods have been tested in interlaboratory studies and sometimes have performance criteria associated with them (see definitions of r , R in section 2.1.1). Therefore, the analyst can find much help from the scientific community but he must still revalidate the method in his own laboratory and establish his own performance criteria. To do so, he will have to validate first within the given context of the very analytical problem all his apparatus used in the analytical procedure. Quality systems such as accreditation or certification of his laboratory oblige him to maintain and verify all basic metrology apparatus: balances, pH meters, thermometers, chronometers, volt or ampere meters, etc.), tools (glassware, ovens, etc.), chemicals (reagents and calibration chemicals), fluids (water, gases, electricity supply, etc.), computers etc. Some other items may necessitate a special validation procedure e.g. spectrometers, software etc.

It must be stressed here that method validation is as important and complex in microbiology. Similar steps and precautions exist. An important part of the book edited by Lightfoot and Maier [6] deals with all these aspects from development and validation of the method, validation of instruments and supplies up to the use of control charts. The reader is referred to these guidelines and to sections 2.3.3 and 2.4.3.

2.3.1. Validation of instruments, computers and software

Instrument validation precedes method validation. The same principle applies for computers and software when automated methods are used. Instrument and computer validation procedures should initially be provided by the suppliers. The analyst has to revalidate them within the analytical procedure under development. It may consist in calibration actions e.g. wavelength (UV, visible or IR) or energy (gamma and x-ray radiations) calibration, noise reduction, temperature controls, fluid throughput etc. It always consists in the proper verification of software used for the calculation of the end results (regression curve algorithms, internal standard peak ratios, etc.), the deconvolution programmes for determining peak surface etc. Software validation is also best done by the producer of the program. He is the person who knows best the access, applied algorithms, and logic of the system. Computer programs must be validated in-house by the analyst within his own method to verify that he obtains the intended results. Having done all that, he can use the instruments for the method development (if starting from zero) or for a direct validation if the method already exists.

2.3.2. Validation of the analytical method

In chemical analysis the property of interest to be determined, e.g. trace elements or organic substances, is rarely directly measurable, as may be the case in physical

analysis (e.g. mass, length, time etc.). To be able to measure the substance or element of interest the analyst has to convert or separate the analyte into a form that is compatible with the detector. Here a detector may be the eye of the operator (e.g. in titrimetry), an electrode, the balance in gravimetry, the thermal, electrical or optical signal generated in a receptor. This may imply that he has to change the physical and/or chemical structure of the initial material but without losing control of the change(s) so that the traceability of the final detection to a predetermined reference (e.g. fundamental units) is not lost.

Validation of a method consists in establishing a number of properties and quantifying performance criteria that will demonstrate that the method achieves the requested performances for a given purpose. These criteria are described below.

2.3.2.1. Full development of a method

In particular in environmental monitoring, unknown and complex samples have to be analysed. It is very common that no standardised method exists for the requested study. No reliable model has been published but only approaching methods are given in the literature. Analytical procedures typically include a pretreatment step, e.g. digestion, extraction, purification or separation, a calibration in case of relative and comparative methods and a final detection. Each action undertaken in one of these steps is a potential source of error that adds to the total uncertainty of the determination. When developing his method, the analyst has to identify and possibly minimise all these sources of errors. To do so he has to work out a strategy, which allows him to study each individual step. One approach is to start from the simplest system which is encountered in the procedure and which corresponds to the calibration solutions to be presented to the detector for the quantification of the signal. Having studied these simple systems he must go towards the real sample. Figure 2.1 shows the main steps of an analytical method for chemical measurements and the steps to be considered for the validation of comparative methods e.g. spectrometric determinations. After a preliminary theoretical investigation (e.g. consultation of literature), the study on solutions of pure substances, he goes for a more complex step, e.g. a digest or an extract, and re-evaluates the performance and adapts the procedure if necessary. Finally, the validation passes to real samples. After each new step the analyst evaluates if the conclusions drawn in the previous step are still valid. If not, he has to come back and adjust conditions. In addition to the reliability of the method the analyst has to evaluate how it will behave in the real situation of daily 'routine measurements' in the hands of the technicians and under economic constraints; he has to estimate the robustness or ruggedness of the method.

2.3.2.2. Validating a standard procedure

In some classical fields of environmental and food analysis which usually undergo regulatory requests, standard methods exist. This is typically the case for water and air analysis, waste disposal or incineration, natural and processed food samples etc. Such standardised methods (in particular the most recent standards) are often described in such a manner that a validation can be achieved rapidly. AOAC has validated hundreds

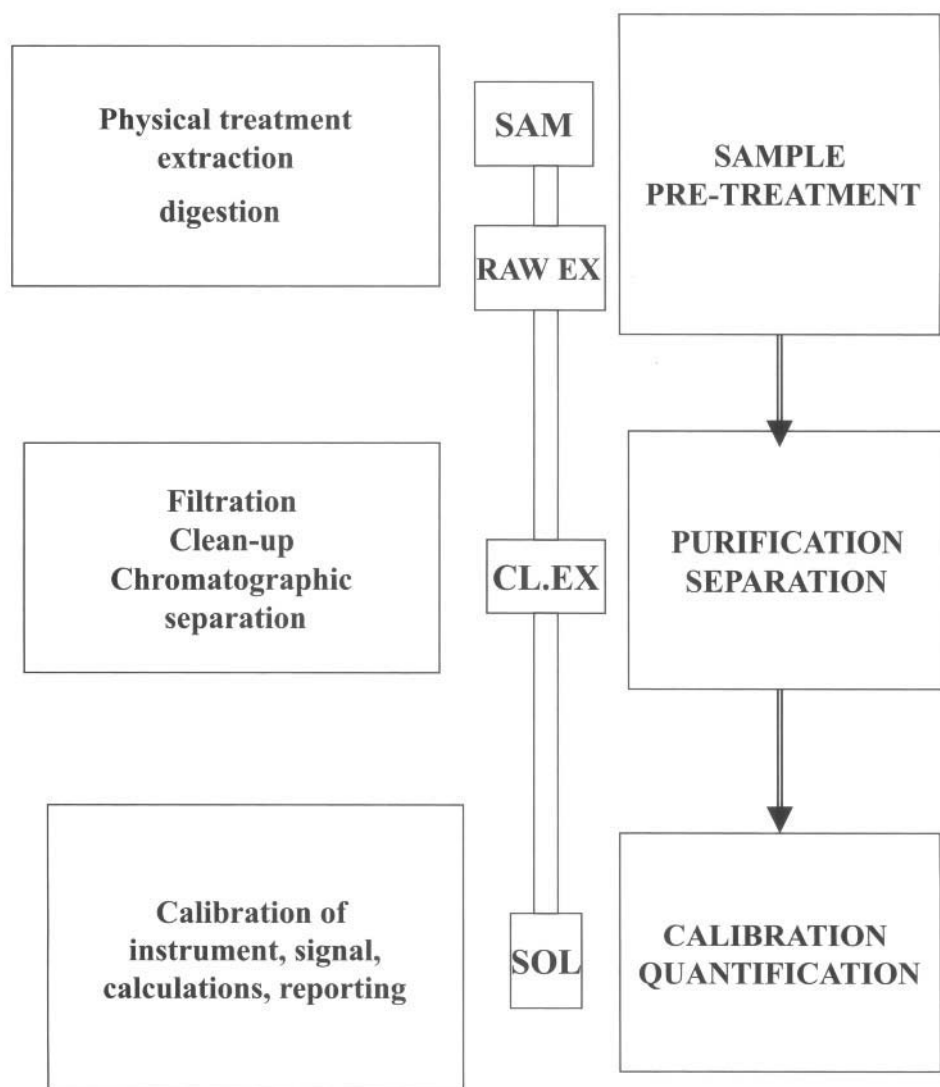


Fig. 2.1a. Main steps of a comparative method in chemical analysis (with the exception of the sampling stage).

In the laboratory, the sample undergoes successive transformations to bring the substance to be determined into a stage compatible with the detection system. Depending on the method one or several of these steps (SAM, RAW EX, CL. EX, SOL) are linked or not existing (e.g. for inorganic determinations the purification step may be reduced to filtering). The validation will consist in evaluating all these steps to avoid any source of systematic error and to reduce and to quantify the uncertainty linked random errors of each step. The principle of the validation is given in Fig. 2.1b.

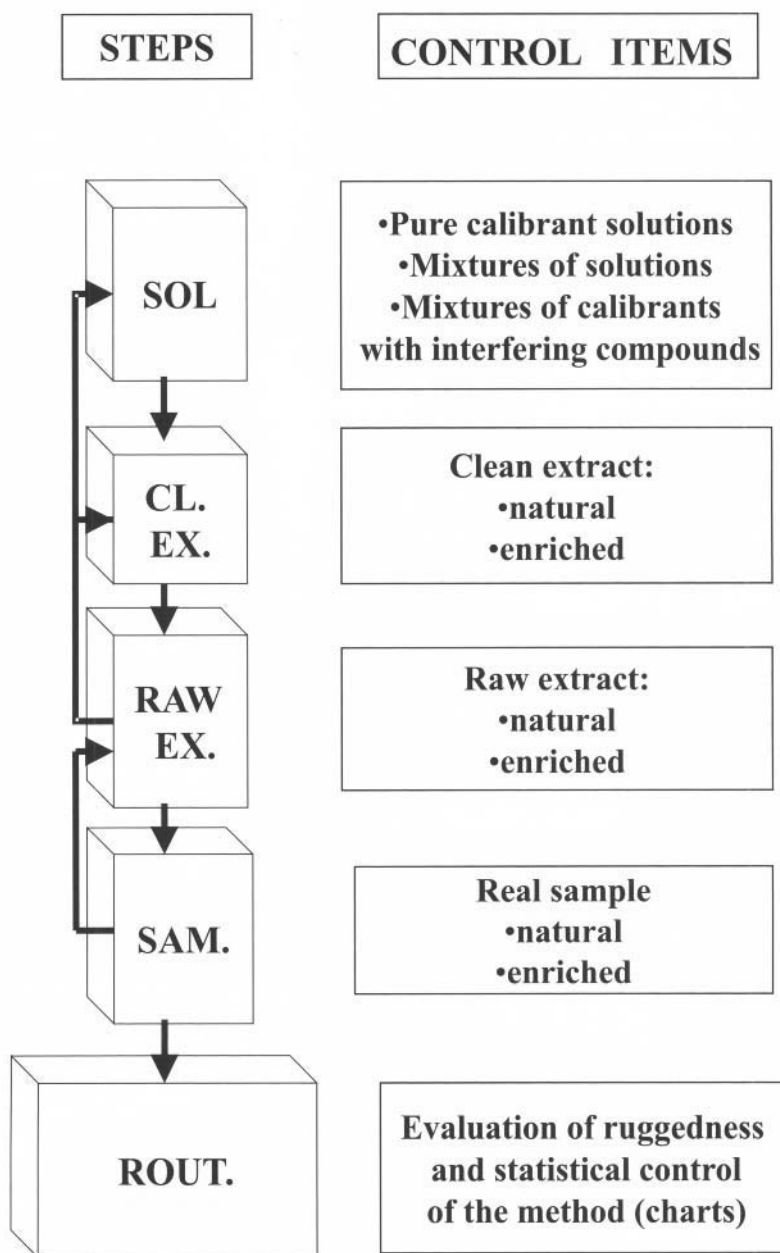


Fig. 2.1b. Step by step approach for the development and validation of a relative (type II) method. The validation follows the various steps followed to set up the method. The analyst starts from the simple systems and stepwise goes towards the situation of the real sample. At each step all critical aspects must be investigated and all sources of errors detected, eliminated and the effect of the random variations quantified. The validation goes the inverse route than the one followed when analysing a real sample as shown in Fig. 2.1a.

of methods and publishes them regularly in a compendium [19]. For the individual laboratory, validation of such methods will consist mainly in the evaluation of the performance criteria within the laboratory. Precision can be measured quite easily, a verification of the trueness will rely on CRMs or adapted interlaboratory studies.

2.3.2.3. Practical examples of method development and validation

To better understand the step-wise approach for method development and validation, it is necessary to give examples. They are taken from organic and inorganic trace analysis of environmental matrices. Figure 2.2 illustrates the steps for the validation of the analytical procedure for the determination of polychlorobiphenyls (PCB) in industrially contaminated soil. Figure 2.3 shows the steps necessary to validate the determination of trace elements and particularly arsenic in a fish tissue. Each step of the procedure will provide the necessary information so that the next step can be done with confidence. In practice, the analyst will develop a procedure to quantify all primary and secondary method characteristics as defined in section 2.1.4.

Literature search

The scientific literature rarely gives descriptions of analytical methods that are sufficiently detailed and validated to be applied directly without risk. Quality control items are usually not given. Therefore, the analyst has to take the general guidelines given, to build up his own system and to evaluate its performance on his own real samples. Also standard methods cannot be transferred directly to other types of matrices without full validation. For example: a standard for drinking water cannot be simply transferred to surface or wastewater. Similarly, a method for sediment analysis is not ipso facto suited for soil or sludge. In our examples, one could consider that articles found in the literature allow one to define in broad terms the pretreatment (acid attacks, extraction solvents), the purification and the final detection systems without giving the details on setting up control points, implementing quality control items, e.g. use of RMs and CRMs etc.

Validation of the final detection

It has to be stressed that errors occurring in the calibration process are very frequent; this has been shown in the discussions held in interlaboratory studies conducted over several years within the BCR. Figure 2.4 shows the outcome of such a study on PCB solutions performed in 1998. The types of mistakes include simple calculation errors, e.g. of concentrations, dilution errors, mistakes during transfer of volatile solvents, use without verification of impure nonstoichiometric primary compounds, contamination, interference, non adapted internal standards or inaccurate introduction of internal standards into sample and calibration solutions, unsatisfactory background correction and blind trust of integration systems, abuse of units (work on a volume basis for the preparation of calibrant solutions and assumption that a *mL* equals a *g*, even for organic solvents or mixtures of acids! handling of ppm, ppb, ppt!), and absence of matrix matching of calibration solutions. Many mistakes have also been noticed on the

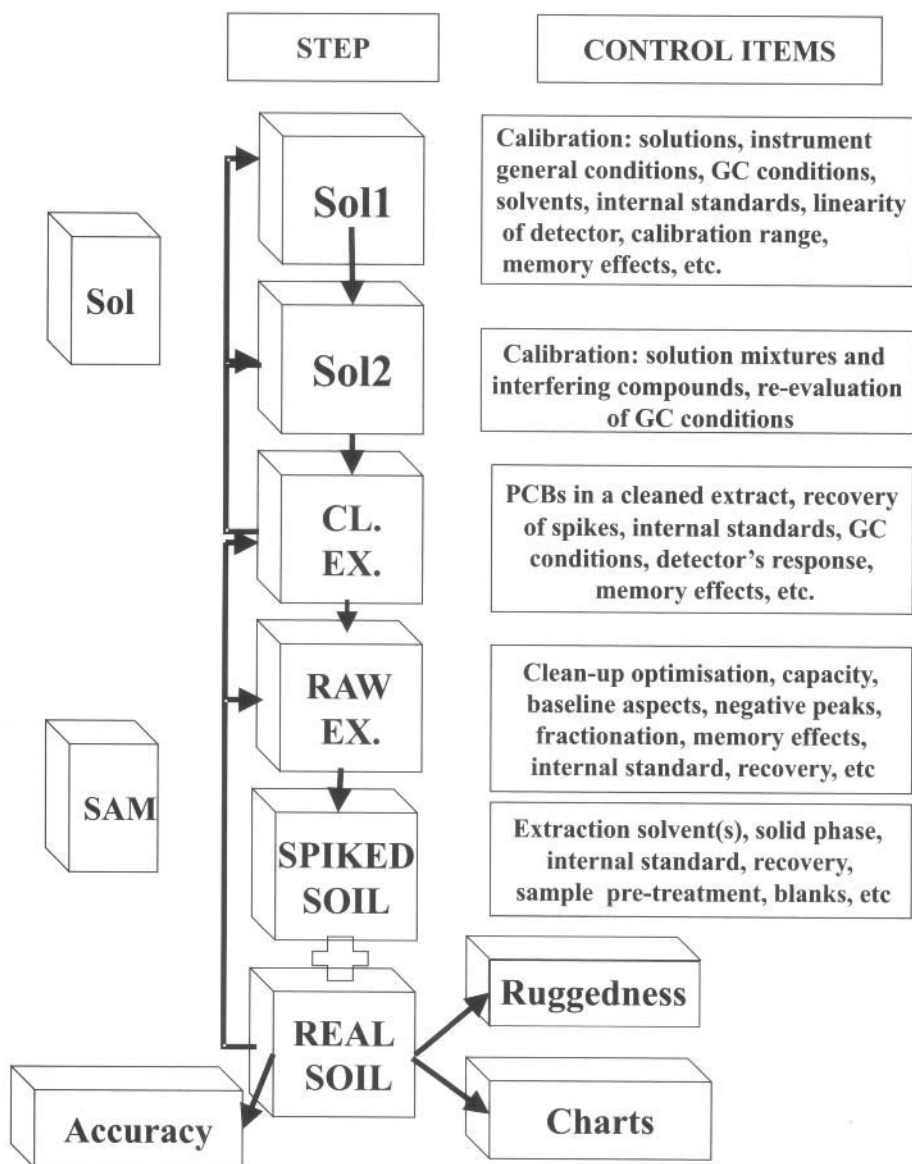


Fig. 2.2. Validation of the determination of PCB in soil.

The validation has the objective to identify, during the method development process, all sources of error and eliminate them or to quantify their contribution to the total uncertainty of the determination. For trace organic determinations particular attention must be given to the quantitative extraction and clean-up of all PCBs. Several types of adapted materials must be prepared to test all steps of the process (from simple calibrant solutions or mixtures, spiked extracts, to spiked soil material). CRMs should be used for validating trueness. Laboratory RMs must be prepared for the establishment of control charts when the method is under statistical control.

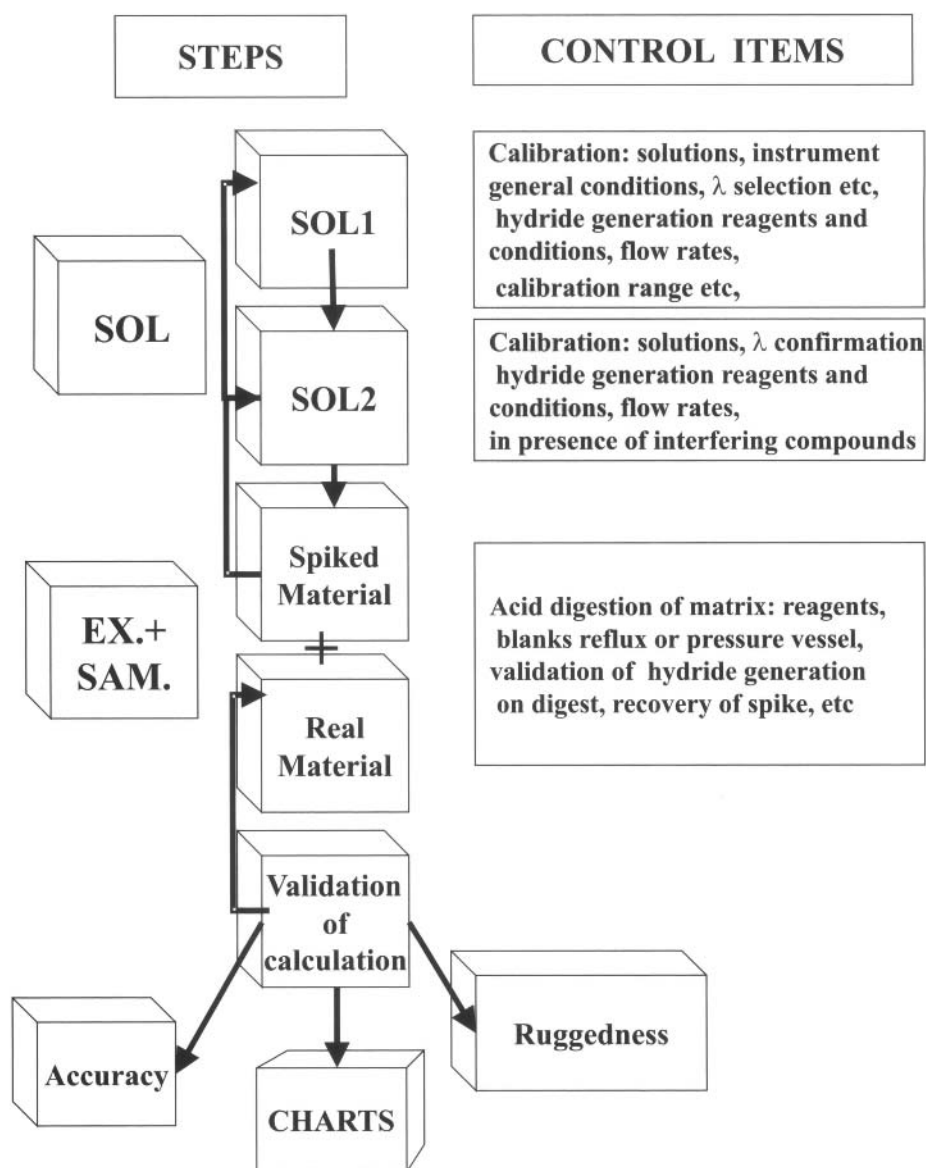
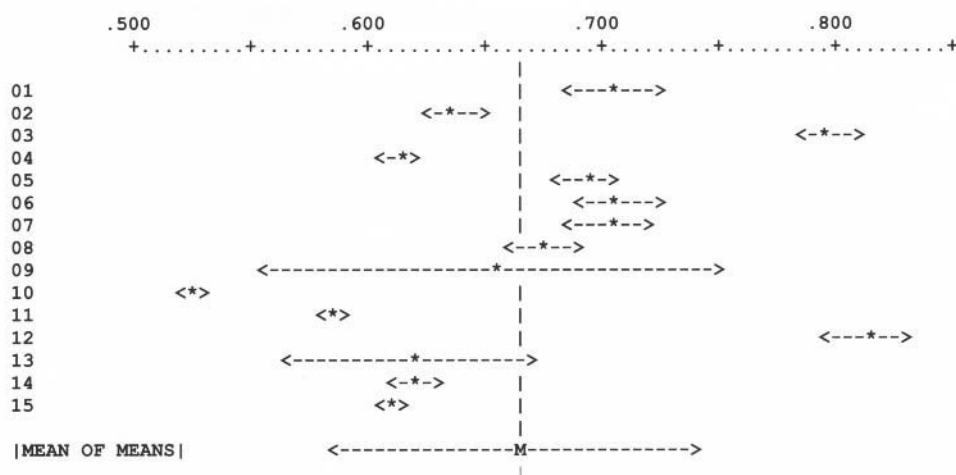


Fig. 2.3. Validation of the determination of As in fish tissue.

The validation has the objective to identify, during the method development process, all sources of error and eliminate them or to quantify their contribution to the total uncertainty of the determination. For hydride generation techniques particular attention must be given to the quantitative transformation of all As species into hydrides (arsenobetaine, arsenosugars etc). Several types of adapted materials must be prepared to test all steps of the process (from simple calibrant solutions or mixtures to spiked fish tissue samples). If they exist CRMs should be used for validating trueness. Laboratory RMs must be prepared for the establishment of control charts when the method is under statistical control [27, 28].



SUMMARY OF RESULTS FOR ALL CONGENERS DETERMINED IN THE UNKNOWN ISO-OCTANE SOLUTION.

CB IUPAC No	Target value µg/g *	Mean of means µg/g n = 3	Between laboratory S.D. µg/g	Between laboratory CV%	Range of values µg/g	Ratio Highest/lowest result
28	0.60	0.66	0.08	12	0.52-0.81	1.56
52	0.61	0.65	0.07	11	0.54-0.80	1.48
101	1.45	1.54	0.12	7.8	1.37-1.75	1.28
105	0.52	0.62	0.11	18	0.45-0.83	1.84
118	1.88	1.92	0.21	11	1.60-2.38	1.49
128	0.45	0.47	0.07	15	0.38-0.63	1.66
138	1.30	1.25	0.11	8.8	1.07-1.46	1.36
149	1.62	1.81	0.17	9.4	1.56-2.26	1.45
153	1.56	1.63	0.11	6.7	1.38-1.78	1.29
156	0.41	0.43	0.08	19	0.27-0.60	2.22
170	0.48	0.55	0.21	38	0.43-1.29	3.00
180	0.59	0.60	0.05	8.3	0.52-0.69	1.33

* obtained on a mass basis

All mean of means (within one standard deviation) cover the target value except for CB 149. In addition to the determined CB the following congeners were also present in the solution, in similar concentrations as the CBs to be measured, in order to create difficulties in the chromatographic separation: CB 31, 44, 47, 49, 56, 66, 85, 87, 97, 110, 137, 141, 151, 187, 194, 202, 206.

Fig. 2.4. Interlaboratory study on the determination of chlorinated biphenyls in iso-octane. Bar-graph for laboratory means and standard deviation of CB 28 for three replicates

way calibration curves are applied. In particular, when multi-elemental or multi-residue techniques are used, often the calibration range does not cover the concentration encountered in the test sample. Sometimes extrapolations are performed in non-linear ranges of the detector's response (e.g. AAS, ECD), standard additions are made at levels where the detector's response is already saturated. Chemometric tools are often used blindly (built in the spectrometer and never tested), or without a critical eye. Errors at the calibration level can often result in differences between laboratories of some orders of magnitude [20].

A study of the detection system with a solution of pure compounds is necessary to detect, evaluate and solve all problems related to the reliability of the signal produced by the detector: specificity, linearity, traceability to adequate pure substances, sensitivity, trueness and precision of the calibration. Figure 2.5 gives an example of a linearity plot of an ECD. All problems of interference, chromatographic separation, choice of the internal standard for quantification, and chemometric tools for a reliable calibration should be solved at this stage. For this step, the analyst will prepare stock solutions of calibrants with known purity and stoichiometry. Choice, handling, storage and preparation of such solutions is described in detail by D.E. Wells et al. for PCB and other organic compound determinations [21] and by J.R. Moody for inorganic calibration [22]. Some of their basic recommendations can be recalled here. Calibrants and calibration stock solutions should be kept in closed storage facilities (e.g. safe, refrigerator, deep-freezer) and their access should be controlled and a responsible senior analyst should be designated for the preparation and distribution. For organic compounds, which are often dissolved in volatile solvents, sealed vials (e.g. glass ampoules), are preferred. Protection from light (e.g. amber glass vials) and from increased temperature (refrigerator, deep-freezer) should be guaranteed. Visual inspection of inorganic and organic standard solutions before each use should allow the detection of important precipitates or flocculates. Before and after taking an aliquot of the standard the analyst should weigh the vial to detect losses due to evaporation or leakage. Stock solutions should be replaced regularly, the shelf life depending on the stability of the compounds and the frequency of use. Some of the discrepancies between laboratories and towards the target value are shown in Figure 2.4.

The study of the detector's response with solution(s) can be subdivided into individual steps with growing complexity. This is interesting in particular for complex procedures encountered in organic trace analysis [23,24]. For inorganic trace analysis, calibration solutions should be prepared preferably from pure metals or oxides rather than salts [22].

Matrix influence

In this step of the validation it is investigated whether the matrix influences the signal of the detector. The study concerns either an extract or a digest; this new matrix may influence parameters defined in the previous step. All the conclusions obtained in the first step have to be verified (calibration, linearity, chromatographic conditions and performance, internal standard etc.). For the determination of trace organic contaminants this step is of great importance as it has to assure that no interfering compounds remain because quantification is often performed with non specific detectors (e.g. ECD, FID,

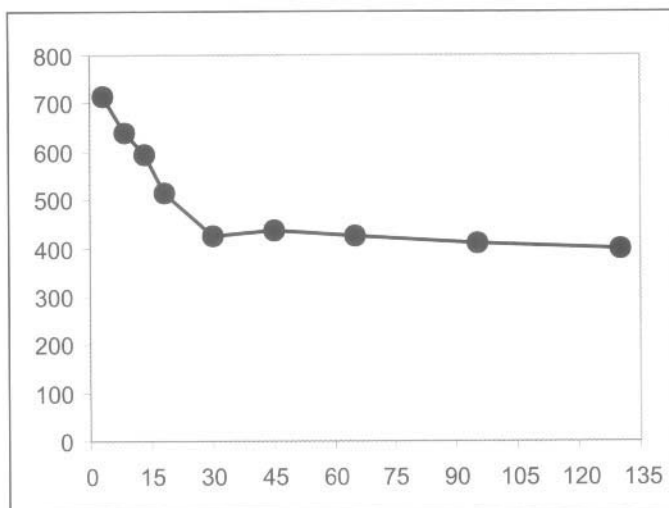


Fig. 2.5. Linearity of the ECD detector.

The graph shows that there is no proportional response of the ECD detector over the entire range of masses tested. (Y axis: peak height/mass CB injected; X axis: mass of CB injected).

UV). Consequently the reliability of the signal relies on the prior chromatographic separation. Matrix matching of the calibration solution or standard addition procedure can help to overcome matrix influences. An example of the too often neglected matrix effect is given in Table 2.1. These results were obtained in an interlaboratory certification study of trace elements in soils. It demonstrates the importance of matrix matching for the calibration [25]. Except for Co and Cu, for which another source of error remained, the matrix matching of the calibrant solutions allowed the laboratory to come closer to the certified values.

In organic trace analysis it is usually necessary to clean the extract before the real separation by chromatography can be performed. The clean-up should remove all co-extracted bulk material such as lipids, sulphur, pigments etc. and other potentially interfering compounds. A proper clean-up also will protect the chromatographic column and the detector from contamination (ionisation source of the MS, ECD). The clean-up has to be sufficient to bring onto the chromatographic column a solution that does not alter the column capacity and does not contain any substance that may influence the detector response. Figure 2.6 gives an example of insufficient clean-up of animal feed material where many negative peaks, due to remaining fat components, influence the determination of the chlorinated pesticides. For the determination of organic traces it is also essential to estimate possible sources of losses due to clean-up. This may be done by spiking of a raw extract. Standard additions will allow one to estimate the capacity of the clean-up and therefore help to define the routine working range. Here the analyst may also decide to select an internal standard to follow the clean-up recovery.

TABLE 2.1

ILLUSTRATION OF THE INFLUENCE OF MATRIX MATCHING FOR INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION AS METHOD OF FINAL DETERMINATION (MASS FRACTIONS IN mg kg^{-1}) OF TRACE ELEMENTS IN A LIGHT SANDY SOIL — BCR CRM 142R

Element	Results without matrix matching mean \pm SD	Results with matrix matching mean \pm SD	Certified value mean \pm 95% CI
Co total	6.0 ± 0.2	7.9 ± 0.5	5.61 ± 0.31
Co aqua regia	4.6 ± 0.5	6.1 ± 0.5	NC
Cu total	607 ± 9	667 ± 18	696 ± 12
Cu aqua regia	655 ± 4	745 ± 12	707 ± 9
Mn total	139 ± 2	151 ± 5	156 ± 4
Mn aqua regia	122 ± 2	151 ± 4	NC
Ni total	216 ± 5	249 ± 8	247 ± 7
Ni aqua regia	207 ± 3	266 ± 5	251 ± 6
Zn total	1826 ± 34	2072 ± 47	2122 ± 23
Zn aqua regia	1856 ± 15	2238 ± 26	2137 ± 50

95% CI: 95% confidence interval

SD: standard deviation of five independent measurements

NC: not certified

For Co and Cu other errors than matrix matching remained unidentified by the laboratory.

Solid material

A difficult step of an analytical procedure is the pretreatment, extraction or digestion procedures. In particular for trace analysis in solid materials it is difficult to estimate its efficiency. In both examples chosen above, the starting matrix is a solid: mussel tissue and soil. For the determination of the trace elements in the mussels it is necessary to destroy the matrix by a strong acid attack. With aqua regia, under reflux or in a closed system, the matrix can be entirely decomposed so that the elements Cd and Pb are liberated. But it would be impossible with such an attack to determine As by hydride generation ICP. As demonstrated by Schramel et al. [26] and confirmed in BCR interlaboratory studies and the certification of forms of As in fish tissue [27,28] some metabolised forms of As (arsenobetaine) only slowly generate hydrides (AsH_3). An attack with only aqua regia is not sufficient; strong oxidising (HClO_4 , H_2O_2) agents should be applied. This difficulty for the hydride generation step of As points out a precaution which should also be taken when applying a standard addition procedure to validate such a method. To reflect the real behaviour of the sample the spikes added to the material should be of the same chemical nature as the native compound.

For the PCB determination in soil and in general for the determination of solid materials for trace organic compounds, the extraction efficiency will be improved and assessed in the 'material' step. The analyst has to find the most efficient solvent (single or mixtures to adapt the polarity) to extract in the minimum time a maximum of

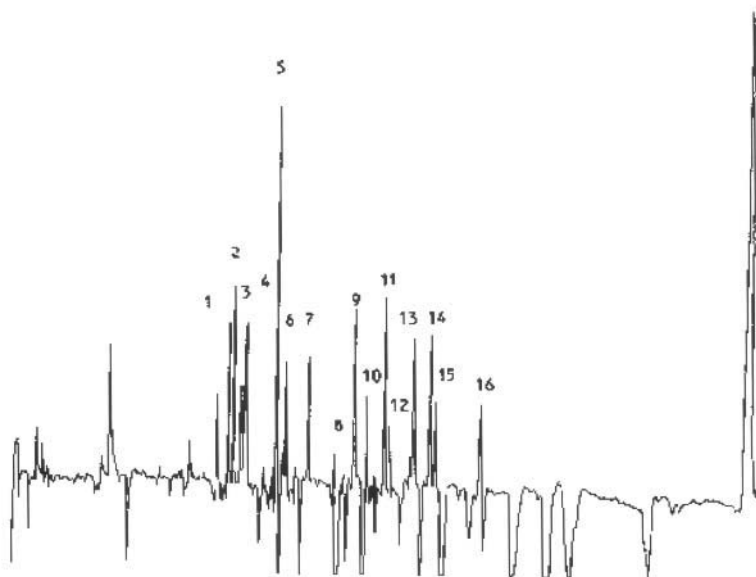


Fig. 2.6. Electron capture detection gas chromatogram of animal feed containing organo-chlorine pesticides. Insufficient clean-up did not allow the removal of all fatty substances of the matrix. These substances affect the detector's response and appear as 'negative peaks'. Co-elution of a pesticide with such a substance leads to an inaccurate determination. 1: α -HCH, 2: HCB, 3: β -HCH, 4: γ -HCH, 5: internal standard, 6: heptachlor, 7: aldrin, 8: β -heptachlorepoxyde, 9: γ -chlordane, 10: α -endosulfan, 11: p,p'-DDE, 12: dieldrin, 13: endrin, 14: p,p'-TDE, 15: o,p'-DDT, 16: p,p'-DDT (adapted from ref. [51])

compounds of interest without overloading the extract with matrix constituents. Usually, the operator favours large and complete extraction rather than a specific but incomplete system and relies on the clean-up to eliminate interfering compounds and matrix constituents (e.g. lipids). Some pretreatment procedures to favour a more specific extraction are possible. For PCBs an attack with potassium hydroxide in ethanol at increased temperature allows one to destroy triglycerides or waxes. If the heating is prolonged the hydrolysis also induces losses of highly chlorinated CB (e.g. CB 180). The extraction of some chlorinated pesticides (except the 'drins') can be improved by a pretreatment of the sample with concentrated sulphuric acid [29].

The validation of the extraction step is difficult and often unsatisfactory. For solids it is nearly impossible to prove that all the compounds have been extracted. Two approaches are usually applied. One is based on successive extractions with fresh solvents and determination of the residual traces of PCBs in the successive extracts. When no PCBs can be extracted any more one may assume (but not guarantee) that the extraction is complete. The second approach consists in a standard addition procedure with increasing spikes [29]. A more detailed discussion on this issue is given in Chapter 5.1.4. After extraction the analyst verifies that he recovers all added compounds. This method is only valid if the operator allows the spike to be in contact for a certain time with the material — at least overnight. By repeating the spiking at each level of enrichment the

analyst may estimate the reproducibility of the extraction procedure in addition to its efficiency. However, this method does not give a guarantee for a full extraction. Since several years alternative extraction techniques have been available, e.g. supercritical fluid extraction, microwave or ultrasonic assisted systems etc. These alternative techniques may help to evaluate the extraction efficiency of simple solvent (Soxhlet) systems, if they are available in the laboratory!

Uncertainty budgets — statistical control

When all major sources of errors are eliminated from all steps of the procedure and only small random variations remain, the laboratory produces results that can be considered as being under statistical control. This means that it can be predicted that results produced on the same reference sample in reproducibility conditions will remain within a certain limit of precision. Having reached this stage, the analyst can set up a statistical control scheme, which verifies that these conditions are maintained. Such systems, which have been developed for the control of production lines, have been adapted to analytical chemistry; they are known as control charts (see section 2.4).

Table 2.2a (validation of a method used for the study of the stability of a CRM) shows a way of estimating the uncertainty of the various steps of a procedure for trace organic substances, and Table 2.2b details an uncertainty budget for the determination of As in a copper alloy by ICP-TOEMS. The inventory and quantification of all uncertainties of the analytical procedure is called an uncertainty budget. Each major step of the procedure has an associated uncertainty obtained by measurement. The total uncertainty of the method is the sum of all these random uncertainties including possible systematic errors e.g. impurity figures of calibrants. In reality it is extremely difficult to establish a real uncertainty budget. It is feasible for primary or calculable methods, very rarely possible for relative methods and never for organic trace analysis. Therefore, the evaluation of the real potential of such methods can only be obtained through an interlaboratory approach. Nevertheless, the analyst must make the effort to estimate as far as possible all the uncertainty contributions of the method in his validation study. The discussion on homogeneity testing of CRMs (section 4.3) gives some details on such uncertainty calculations.

As said before, working under a strict quality assurance system minimises many sources of errors as it obliges one to organise a verification of all basic metrology tools within the laboratory. One of the most often used tools is the balance. It serves to calibrate glassware, analytical tools such as pipettes and burettes but also to prepare calibration solutions. In environmental trace analysis, when properly used, gravimetry represents a component of the total uncertainty that is far smaller than those due to the pretreatment and final quantification of the procedure. Therefore, the analytical chemist often estimates the total uncertainty of his measurements by simple replicate determinations on the same sample. The repeatability figure, r , is however an underestimated figure of the real performance of a method. A better estimate would be given by a reproducibility figure, R , obtained from different independent laboratories. Except in standardisation studies or method development studies like those performed by the European Commission within the SMT programmes (BCR) or bodies like AOAC, IDF etc., this value R is rarely available.

TABLE 2.2a

VALIDATION FIGURES OF VARIOUS STEPS OF THE DETERMINATION OF CB IN FRESH HOMOGENISED MUSSEL TISSUE

When comparing the data obtained with 5 replicates of three successive steps there is a tendency to increased CV%, but several exceptions can be noted. In the present study the differences between CV are small from one step to the other. This shows also the limit in establishing the uncertainty budget for measurements of complex organic trace substances. This study was performed in the certification of BCR-CRM 682

STEP	Coefficient of variation (CV %) of the various tested steps CB IUPAC No and number of replicates (n)					
	n	CB 52	CB 101	CB 118	CB 153	CB 180
Calibration 1)	5	2.0	1.7	3.7	2.6	4.6
Calibration 2)	10	1.7	2.1	2.9	2.3	3.5
Clean-up 3)	5	3.3	2.9	4.1	2.5	4.3
Total 4)	5	3.5	3.5	3.7	1.3	3.1
Total 5)	5	3.5	3.3	1.8	2.4	5.5
Total 6)	10	3.5	3.7	2.4	2.2	3.6

- (1) determination in 5 replicates of the same solution of CB in one series (one calibration)
- (2) determination in 10 replicates of the same solution of CB in one series (one calibration)
- (3) determination of 5 analytical portions of a raw extract of mussel tissue — 5 clean-up and one calibration for the final determination (one calibration)
- (4) determination of 5 sub-samples (first five) from the same vial of homogenised fresh mussel tissue (one calibration)
- (5) determination of 5 sub-samples (second series of five) from the same vial of homogenised fresh mussel tissue (same calibration as 4)
- (6) total of the 10 sub-samples of 4 and 5 (one calibration)

Check for trueness

When the operator has determined the totality of the uncertainty budget within the possibilities offered by his laboratory, the uncertainty due to the presence of (a) possible systematic error(s) or bias remains. Only one possibility exists to detect such a bias. It lies in external help. Comparing the results of the test method to another method developed in-house involves the risk of having an unknown laboratory bias, e.g. biased primary calibrants etc. Therefore, it is more appropriate to look for external help. This can come from the comparison of results obtained on a reference sample with those obtained on the same sample by another laboratory or by analysing a certified reference material. Both possibilities will be dealt with in the next two chapters of this book.

Quality control points

When all steps of the method have been developed, optimised and verified, the analyst has to combine the procedure and work out quality control items for routine use which may allow him to investigate *a posteriori* that the method is still under control. The control

TABLE 2.2b

UNCERTAINTY BUDGET FOR AS IN COPPER ALLOYS USING ICP-TOFMS AND WITH ^{115}In AS INTERNAL STANDARD (COURTESY OF XIAODAN TIAN AND HAKAN EMTEBORG, UNIVERSITY ANTWERPEN, BELGIUM).

The uncertainty budget was established following the guidelines issued by EURACHEM "Uncertainty in Analytical Measurements" (first edition 1995).

The uncertainty components judged as significant are listed in the table. The general mathematical expression used to calculate the final concentration is:

$Y = [(S/k) \times V_{in} \times D_{f1} \times D_{f2}] / M$ where S is the instrument signal, k the slope of the calibration line, V_{in} is the initial volume of sample (corrected for density), D_{f1} and D_{f2} the dilution factors applied, and M the mass of solid sample used. Measurements were done in the ion counting mode and results obtained on the bases of ratios of $\text{As}/^{115}\text{In}$. Dilutions made on a mass basis. The uncertainty of D_f was calculated as follows: $UD_f = ([uv_1/v_1]^2 + [uv_2/v_2]^2)^{1/2}$. The total uncertainty has also taken into account the uncertainty of the purity factor of the initial calibrants and has been obtained as the square root of the sum of the squares of the individual relative uncertainty (R.U.) components. The uncertainty of k was estimated as negligible as all correlation coefficients were $r^2 = 0.999$.

Step and unit	Value	Uncertainty	R.U. %
As calibrant			negligible
Weighing of solid sample, g	1.000	0.001	0.1
First solution, g	100.000	0.001	0.001
First dilution, v_1 and v_2 , g	48.0	0.001	0.1
Second dilution, v_1 and v_2 , g	50.0	0.001	0.1
As/In ratio by ICP-TOF-MS ion counting	0.0107	0.0004	3.68
Concentration of I.S. in calibrant, ng/ml	20.456	0.380	1.860
Concentration of I.S. in sample, ng/ml	20.0	0.20	1.0
Correction for density, g/ml	1.0405	0.00298	0.286
The combined uncertainty is:			4.26%

points should be linked to those sensitive items he has recognised during the validation. For inorganic determinations, Table 2.3 lists sources of errors which may be subject to control points in the determination of trace elements by destructive (Table 2.3a) and nondestructive (Table 2.3b) methods. A similar listing is given for organic determinations in Table 2.4. Ideal control points are those which are built into the normal analytical scheme and can lead to a fully automated warning e.g. for blanks solvent purity and/or contamination. Other control points may be sensitivity performance checks through the slope of the calibration line, the separation power of a chromatographic system for two artificial substances introduced into the samples and calibrants (e.g. internal standards). The result from the critical control points should be included into the standard reporting form delivered by the analysts so that problems which arise can be traced back. Each situation and each method needs specially worked-out control points. Some standardised methods or methods recommended by EPA or the AOAC [19] include such control points [30]. More and more standards for difficult analytical tasks contain performance criteria for

TABLE 2.3A

SOURCES OF ERROR IN THE VARIOUS STEPS OF DESTRUCTIVE METHODS FOR INORGANIC TRACE ANALYSIS (SEE DEFINITIONS IN SECTION 2.1.1.2)

Analytical step	Type of error	Contribution	Origin of error	Elimination by
Preparation	Weighing procedure	+/-	Non calibrated balance Inaccurate masses	Calibrate balance, contact balance supplier or metrology services
	Volumetric handling	+/-	Non calibrated glassware No temperature control	Dilution etc. carried out with calibrated glassware, temperature control
Moisture	Adsorption/desorption	+/-	Delay in proceeding Moisture in atmosphere	Correction to dry mass, protect from environment
Digestion/fusion	Volatilisation	-	Open heating systems	For volatile elements (e.g. As, Se) treatments carried out in closed systems
	Adsorption/desorption	+/-	Surface activity of roles or tools	Acid washed hard glass containers. PTFE or HDPE pre-rinsed surfaces
	Incomplete attack	-	Fusion/Oxidation reagents Power too low	Pressurised digestion with oxidising acids; residue checked to verify the total digestion of the matrix. Increase fusion reagents
	Contamination	+	Low purity reagents	Reagents of appropriate purity; verification with blank determinations
		+	Tool or vial material not clean or wrong material	Acid washing as appropriate: when contents below 1 µg/g to be determined: steaming; verification by blank determinations
		+	Sample exposed to laboratory air	Use of clean benches or clean room (class 1 for sub ng/g levels); care in performing methods under cover or in closed systems; verification with blank determination

TABLE 2.3A

CONTINUED

Analytical step	Type of error	Contribution	Origin of error	Elimination by
Sample preparation/clean-up/pre-concentration	Adsorption/irreversible precipitation	–	Wrong working pH or too concentrated solution	pH-control dilution and/or addition of complexing agents if necessary (Al, Fe, Si etc.)
	Contamination	+	Insufficient reagents	As above for digestion/oxidation
	Incomplete conversion	–		Excess of reagents; methods verified <i>a priori</i>
Calibration	Calibration solutions	+/-	Matrix effect, work outside linear range of the detector	Reagents of suitable purity and stoichiometry; where necessary verification of stoichiometry and purity of calibrants; different calibration methods when possible; calibration graphs, matrix-matched calibration solutions and standard additions.
	Detectors response	+/-	Detector / sensor contaminated	Clean surface
	Calculation software	+/-	Wrong algorithms or deconvolution program/straight line or curve fitting	Validation of software-wrong peak is used. Verify ion masses in M.S.

+ : overestimation of content, – : underestimation of content

TABLE 2.3B

SOURCES OF ERROR IN THE VARIOUS STEPS OF NONDESTRUCTIVE METHODS FOR INORGANIC TRACE ANALYSIS
(SEE DEFINITIONS IN SECTIONS 2.1.1.2)

Analytical step	Type of error	Contribution	Origin of error	Elimination by
Counting	Interferences	+/	Peak overlap intrinsic irradiation	Deconvolution; selection of proper decay times; RNAA as an alternative, change energy peak in XRF
	High background	+/	Complex sample	RNAA as an alternative, increase counting time, change X-ray source or secondary target
	Geometry	+/-	Wrong position of sample in X-ray or neutron beam, difference between sample and calibrant position	Calibrant and unknowns both in the same form e.g. solution or powder; measured in identical vials at the same distance of the detector; check for chamber background
Irradiation	Self-shielding	+/-	Shielding effect, absorption of X-rays by major components	Verify that the contents of the major or shielding elements does not affect the investigated elements
Calibration	Changes of flux	+/-	Absence of flux monitor (γ spectro.) or check of Compton effect (X-ray spectro.)	Flux monitors added in the irradiation process; matrix matching for major elements in energy dispersive X-ray fluorescence
	Type of calibrant	+/-	Calibrant not adapted	Same remarks as for destructive methods; additional care to be given to the stability on irradiation of the calibrant

+ : overestimation of content, - : underestimation of content

TABLE 2.4

SOURCES OF ERROR IN THE VARIOUS STEPS OF METHODS FOR ORGANIC AND ORGANO-METALLIC (EXCEPT DETECTION) TRACE ANALYSIS (SEE DEFINITIONS IN SECTION 2.1.1.2)

Analytical step	Uncertainty components	Contribution	Origin of error	Elimination by
Preparation	Error by weighing	+/-	Non calibrated balance Inaccurate masses	Calibration by accredited body
	Volumetric handling	+/-	Non calibrated glassware No temperature control	Work on a mass basis Calibrate glassware
Moisture	Absorption/desorption	+/-	Delay in proceeding Moisture in atmosphere	Protect sample from atmosphere Proceed quickly correct for dry mass
Extraction	Solvent polarity	-	Not adapted to target substances Produces emulsions	Change solvent, use mixture of solvents
	Particle size of solid matrix	-	Clogging, bad contact between solvent/solid particles/ substances Surface activity of solids/particles	Rewet solid, change solvent, add excipient to solid, pretreat with acid etc . . .
	Solvent purity	+	Contamination	Bi-distil solvent, change to higher purity grade, change solvent
	Extraction vessel	-	Contaminated Reflux malfunctioning	Clean or replace, produce a blank, verify reflux, adapt water pressure
	Extraction conditions	+/-	Decomposition of substances	Lower temperature of extraction system
		-	Incomplete extraction	Prolong Soxhlet cycles, increase or lower temperature, prolong heating time, break emulsion, adapt microwave oven
	SFE conditions	-	Flux of supercritical fluid or polarity unadapted. Losses in trapping device extraction	Add MeOH or other polarity modifier Adapt flux and contact time Change trapping device

TABLE 2.4

CONTINUED

Analytical step	Uncertainty components	Contribution	Origin of error	Elimination by
Clean-up	S.P.E. conditions	–	Incomplete by saturation or inadequate affinity of solid phase, sample flux to high	Change disks or columns. Change phase. Lower sampling time. Reduce flux of sample
	Adsorption/chemical reaction	–	Ageing of phase, water activity too low or high	Change phase, use mixed beds or layered sorbents
	Complex matrix	–	Presence of S	Eliminate with Cu wire or C phase or AgNO ₃
Derivatisation	Contamination	+	Sorbent quality	Change batch or supplier
	Incomplete conversion	–	Insufficient/Inefficient reactant	Change reaction conditions or reagent. Prolong time. Use method without derivatisation
Separation (LC)	Calculation/software		Wrong algorithms/deconvolution programs/straight line or curve fitting	Validation of software. Wrong peak is used. Verify ion-masses in M.S.
	Co-elution of substance	–/+	Inadequate elution. Solvent type/gradient/ flux/phase inadequate	Changing columns and elution conditions (solvent mixture, isocratic/gradient, temperature of column, phase etc.)
	Retention / transformation Poor peak shape	–/+	Inadequate or degraded phase	Change phase
Separation (GC)			Inadequate conditions; column saturated	Adapt injection volume and quantity of substance injected
	Co-elution of substances	–/+	Inadequate phase / gas/temperature conditions too short column	Change phase or column conditions (more theoretical plates) or gas and temperature conditions. Use two different phases to verify absence of co-elution

TABLE 2.4

CONTINUED

Analytical step	Uncertainty components	Contribution	Origin of error	Elimination by
Separation (CZE) Calibration / Separation techniques	Retention / degradation	-/+	Injection system degrades substance; altered column, saturated column	Change injection temperature, and volume, change inlet, change column
	Absence of separations	-/+	Ionic force and composition of mobile phase	Adapt composition of mobile phase, buffer, current conditions
	Detectors response	+/-	Work outside linear range Saturation/under detection limit Contamination of detector	Lower/increase sample intakes or concentrate Dilute sample or/and calibration samples Run blanks
	Negative peaks	+/-	Co-elution of fatty substances	Improve clean-up
	Calibration solutions	+/-	Substance purity, old solutions, error in dilution. Poor internal standard(s) Contaminated solvent	Change calibrant solutions Check purity of substances in concentrated solutions Correct for impurities Change supplier of substances/ internal standard/ serial dilution Change solvent or redistill

individual control points. In such cases, the effective follow-up of the control points takes place on known reference materials, which are included in the sample sets.

Robustness, ruggedness

A method can only be applied in a testing laboratory when it is sufficiently robust. Slight variations in the method should not affect its reliability:

- small variations of temperatures during pretreatment, ageing of LC or GC columns with decreasing separation power, ageing of graphite tubes in GFAAS, variations in the water content after drying, etc.;
- replacement of parts of the equipment (e.g. Soxhlet, digestion bomb, etc.);
- other technician;
- small variations in the sample matrix, concentration range of the substance, size of particles (e.g. two soils are never the same), etc.;
- variations in the environment, e.g. temperature, humidity, atmospheric pressure.

All parameters fixed during the development and the validation procedure should be investigated for the effect of small variations. The totality of the method ruggedness (influence of the operator) needs to be evaluated on the total procedure by modifying some of the parameters that have been identified as critical during the stepwise approach. A too sensitive method, towards variations, may be inadequate. Ruggedness testing should always be part of the development of standardised methods and can be estimated in interlaboratory studies. Chemometric tools [7] and expert systems have been proposed to estimate the ruggedness of a method.

2.3.3. Quality control of microbiological determinations

2.3.3.1. Microbiological methods

Microbiological analyses are based on the cultivation of microbial populations in artificial environments (culture media) under laboratory conditions. Most micro-organisms will grow if there is sufficient carbon (C) and nitrogen (N) present and will increase in numbers by simple division into two. In liquid media growth becomes visible because the medium becomes turbid. Thus, the presence of only one micro-organism can result, after incubation for a certain time at a specific temperature, in a completely turbid medium, because of the bacterial multiplication.

When the growth medium is solidified (by the addition of gelatin or agar-agar), it is possible to determine the original number of micro-organisms in a sample, as each viable organism or small group of organisms within the sample will grow to produce a large concentration of organisms (colony) which is visible by eye. Such individual organisms or small groups of organisms are thus referred to as colony-forming particles (cfp), often called colony-forming units (cfu).

The methods used for the microbiological analyses of water and food are designed to indicate the degree of contamination and hence the safety and acceptability of the product for consumption. Many of the tests in water and food microbiology are based on the enumeration of the so-called indicator organisms. A direct search for pathogens in food and water is mainly hampered by two problems:

- (1) the number of cfp is generally very low and their distribution may be extremely uneven particularly in solid materials, such as food [31,32];
- (2) the methodology for their detection is often inadequate, hence negative results are often of poor reliability.

Depending on what information is required, the contamination level of the sample and/or the presence of certain organisms can be determined with different media:

- (a) non-selective media — these will allow the growth of many types of micro-organisms and will give information on both the presence and/or level of micro-organisms present;
- (b) selective media — these are formulated so that they contain ingredients which will only permit the growth of certain organisms while all or most of the others present in the sample will be suppressed; they will give information on the presence and/or level of the target organism;
- (c) confirmatory media — these are mostly used after pre-culture on selective media; typical colonies, of the target organism in or on the selective media, are confirmed, in or on these media, as being the required target organism;
- (d) identification media — these are groups of selective media that can provide a more detailed identification of the target organism, first isolated on a selective medium.

Whether the target organism will grow on a medium depends on the composition of the medium, but also on the vitality of the bacteria. Many bacteria are often sub-lethally injured, because for example of lack of nutrients in their natural surroundings, or because of external stress, caused by heat processing of food. Such micro-organisms will grow more easily in non-selective rich media than in selective media. Some selective media are therefore combined with a resuscitation step, using for example a non-selective medium, or pre-incubation at a lower incubation temperature.

The medium, which will be used for the detection of the target organism in the sample, is always a compromise. The choice strongly depends on the type of sample to be analysed. As shown in Figure 2.7, highly selective media will usually give low recovery of the target organisms (as well as smaller colonies), but also low interference of the non-target (competitive) flora. On the other hand, less selective media will give rise to a higher recovery of the target organism, but also of the non-target organisms and therefore more interference.

For the detection of different organisms, qualitative, semi-quantitative and quantitative tests are available. The principle of these tests is given in Figures 2.8 a, b and c. For all these tests, incubation for a certain time at a specified temperature is necessary for multiplication of the target organism(s).

Qualitative tests:

Presence/absence tests: a known quantity of the water or food sample is inoculated into a liquid medium. The presence of particular organisms may be shown by turbidity and/or gas production or alteration of the colour of the medium etc. but can often only be confirmed by culture onto selective solid or confirmatory (solid or liquid) media.

Semi-quantitative tests: Most probable number (MPN) tests: These are based on the presence/absence test. Decimal dilutions of the test sample are inoculated into a set number of tubes of liquid media. Bacterial densities can be determined from

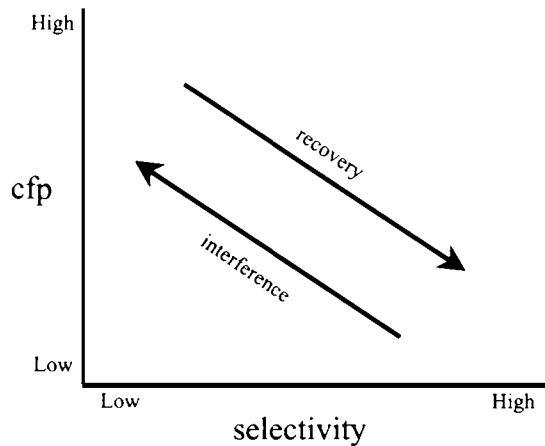


Fig. 2.7. Selectivity and recovery of microbiological methods. The more selective a method (in particular the culture medium composition), the less competitive microflora will grow but also injured bacteria will form no colonies or only tiny ones (cfp: colony-forming particles). Microbiological skill will be based on the ability to select a method with the smallest interferences, the highest recovery for a given type of sample [4].

the combination of positive and negative tube results read from a probability (MPN) table.

Quantitative tests:

For these tests a known quantity or decimal dilution of the sample is used in combination with a solid selective or non-selective medium.

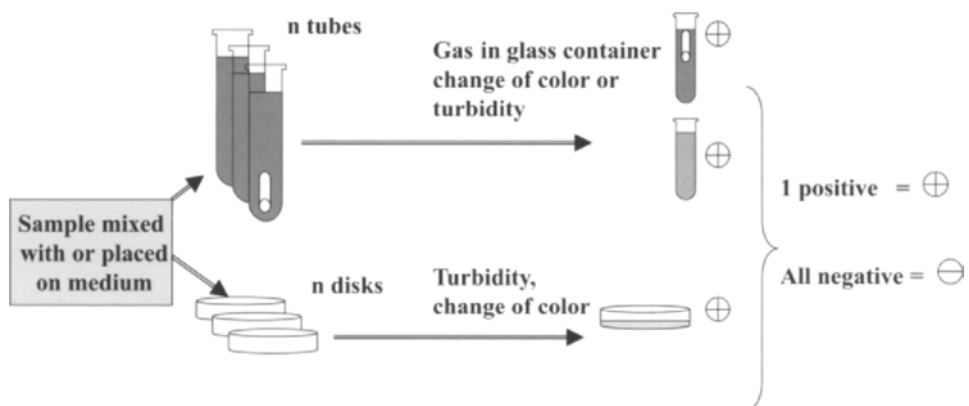


Fig. 2.8a. Principle of microbiological tests. Qualitative tests.

Any positive tube or plate signals a positive result. The more tubes or plates used, and the larger the samples, the more the test is reliable.

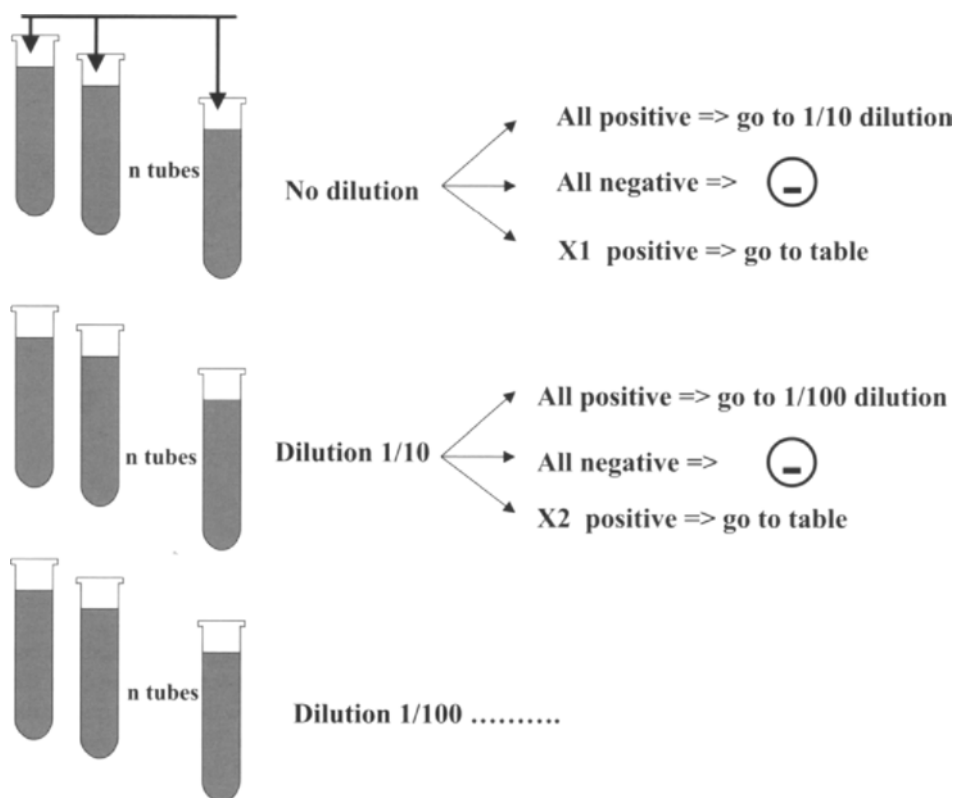


Fig. 2.8b. Semi-quantitative methods or Most Probable Number (MPN).

Tubes containing the culture medium are inoculated with a pure or diluted sample. The analyst evaluates the number of tubes which are positive at each dilution. Positive tubes usually change colour or gas is produced in the tube. The more tubes used, the better the statistical evaluation of the contamination level will be. The number of tubes rapidly becomes a limiting factor due to workload. With miniaturised tubes (e.g. Microtiter® plates developed by the Institut Pasteur uses 96 tubes for 8 dilutions) the MPN method becomes economically affordable. When all tubes for a dilution are positive the result is deduced from the next dilution. For very contaminated samples or very clean samples the MPN method becomes a real qualitative method.

Pour plate method. An aliquot of the neat or diluted sample is added to a sterile Petri dish and mixed with the selected molten agar medium. When the agar has solidified, the plates are incubated for a predetermined time at the specified temperature. Surface or subsurface colonies will develop in some of the agar plates, which can be counted to provide a quantitative value for the bacterial density of the original sample. They can also be picked for further qualitative study.

Spread plate method. A pre-selected small volume of the neat or diluted sample is inoculated onto a solid selective or non-selective medium. It is spread uniformly over the surface by mechanical or manual methods e.g. by holding a sterile stick at a set

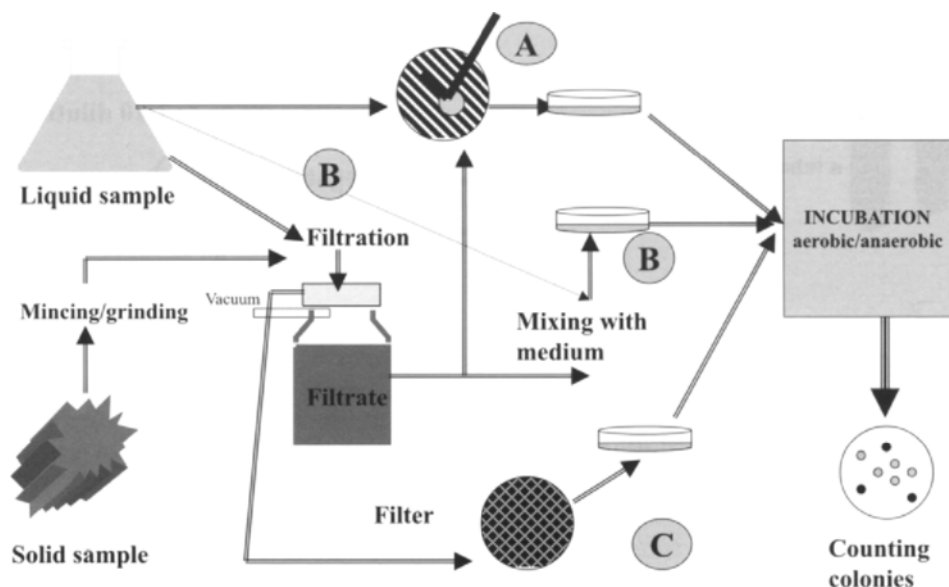


Fig. 2.8c. Quantitative methods of the determination of micro-organisms.

Liquid samples can be analysed directly by the spread plate (A) or the pour plate method (B) but they can also be filtered to retain the micro-organism on a filter which is then placed on the culture medium (C). Suspensions need to be filtrated or centrifuged: afterwards the filtrate or the filter can be analysed. Solids (e.g. food) must be minced before filtration. Incubation at a given temperature can be aerobic or anaerobic. The result of the counting will depend on the ability to isolate the target organisms with the best recovery rate.

angle on the medium and rotating the plate or stick until the inoculum is distributed evenly. After incubation the colonies which developed can be counted.

Membrane filtration method. This method is widely used for water but has some applications for food examination. A certain volume of the water or diluted food sample is filtered through a membrane filter. The filter is placed on the surface of a solid medium (usually selective). After incubation for the selected time and temperature the bacteria retained on the surface of the membrane develop into visible colonies, from which a value for the number of cfp in the sample can be calculated.

If the number of target organisms in a sample is too low to be detected directly with one of these tests or if their growth is suppressed by competitive (non-target) micro-organisms, it is necessary to carry out a pre-enrichment culture of the target organisms in a selective broth. This will never be a quantitative test, but more a presence/absence test.

In the last decade, great effort has gone into the development of new, more rapid, detection methods. These methods are among others based on DNA and immunological techniques, but most of them are not yet routinely used in food and water microbiology laboratories.

2.3.3.2. Accuracy and precision of microbiological tests

In this section the terms accuracy, systematic error and precision are defined as proposed by Havelaar et al. [4]. These concepts are derived from quantitative analytical chemistry but can just as well be used in quantitative microbiology. According to ISO/DIS 6107-8 [33], accuracy is defined as the degree of similarity between the measurement and the 'true value' of the measured quantity. In a microbiological context the 'true value' is the number of colony-forming particles in the sample. The accuracy is related to the overall error of measurement, that is the difference between the measurement and the 'true value' of the measured quantity. The overall error can be divided into three different components:

Firstly, there is the *systematic* deviation between measurement and true value that cannot be avoided with the characteristics of the sample being analysed. Secondly, there is a deviation that is *typical for a laboratory*, arising from the peculiarities of that laboratory. Thirdly, there is a *random error* that is made at a particular instant that would be different when the laboratory repeated its measurement. In summary, the model for the result of a single measurement is:

$$y = \mu + (m - \mu) + B + e = m + B + e$$

where μ is the hypothetical true value; $(m - \mu)$ is the systematic deviation or bias; m is the outcome averaged over a large number of laboratories; B is the mean deviation of a particular laboratory from the overall mean and e is the random error made at a particular instant within a laboratory.

The parameter e must be reduced within the laboratory by elimination of all avoidable mistakes, by training the personnel and by good management. The magnitude of e can be assessed by intra- and interlaboratory studies. The magnitude of B can only be assessed by participating in interlaboratory trials. The variances of B and e together determine the 'reproducibility' (between laboratories). The variance of e determines the repeatability (within one laboratory). Together they are referred to as the *precision* of a method. Assessment of the systematic error is, unfortunately, very difficult, because the 'true value' of the measured parameter is never known. A method that can be trusted to deliver the 'true value' in all circumstances does not exist. The accuracy can be approached by applying several methods by experienced laboratories. It is assumed that the biases will average out. The accuracy can also be assessed by analysing a certified reference material, similar to the unknown sample. Conversely, certification of a reference material depends on the existence of reliable methods.

In section 2.3.3.1 the existence of methods giving a presence/absence result are mentioned. With the results from such tests a breakdown of the overall error becomes quite complicated. Only two types of error can be clearly discerned, either the method incorrectly identifies a sample as positive (false positive) or it incorrectly identifies a sample as negative (false negative). A method is fully accurate if it avoids both types of error.

2.3.3.3. Validation of microbiological methods

The above detailed sources of errors also indicate where the validation of the method has to be implemented. False negatives represent the major unacceptable error in microbiology in terms of potential health and safety consequences. False positives may have an economic impact as they can lead to unnecessary rejection of products. In microbiology, the result relies mainly on the culture medium aspects which have the objective to allow selective growth of the microbe of interest and to highlight a specific property to differentiate it from competitive microflora (aspect of the colonies, biochemical properties etc.). No calibration aspects are involved in microbiological measurements. Counting may be difficult in cases of overgrowth on the plate or because of difficulties to differentiate the microbe from the background flora. These two aspects, beside medium composition, depend mainly on the culturing environment: temperature of incubator, time, light, anaerobic or aerobic conditions etc. Validation consists in the verification of the instruments and consumables used. Lightfoot et al. have given details on how to validate all aspects of microbiological methods in their guidelines for food and water microbiology [6]. These guidelines should be consulted for more detailed information.

2.3.3.4. Quality assurance and standardisation

To improve the quality of the microbiological analysis of food and water most attention has been directed towards the formulation of standardised methods and the development of criteria for culture media, critical reagents, membrane filters and the essential basic materials.

Quality control of basic materials such as media and reagents is mainly carried out by the manufacturers. Each will use their own methods, test strains and criteria and will thus add to the variability of the results obtained with different products. There are, however, some efforts being made to standardise these tests at an international level, for example the 'Working Group on Culture Media' which operates under the auspices of the International Committee on Food Microbiology and Hygiene (ICFMH). Standardisation of methods can be obtained in various ways. At a national level it can be imposed by government regulations. Many countries have organisations involved in the development and implementation of such methods e.g. NNI (Netherlands), DIN (Germany), BSI (United Kingdom), AFNOR (France).

Internationally, standardisation of methods has mainly been carried out within the framework of the International Organisation for Standardisation (ISO) although other organisations such as the Comité Européen de Normalisation (CEN) or the International Dairy Federation (IDF) for dairy products are also actively involved. The choice to standardise a method is not always sufficiently based on comparison of methods or on performance characteristics. Acceptance of international standard methods takes considerable time as all countries or states involved have to agree on the proposed method. Despite the availability of a great number of internationally accepted methods there are still important differences between the national methods used in EC member states. There are namely situations for which a national method is optimised for a local

situation or a type of sample and therefore not valid for other countries. Hence, continued adherence to national standard methods can be attributed to 'tradition' but also to technical reasons. Most of the ISO microbiological methods give no guidelines for quality control of the methods; an exception is the revised ISO standard method for the detection of *Salmonella* [34] in which the use of positive control samples is recommended.

The use of different laboratories can give rise to results that are not comparable. Van Schothorst et al. [31] showed that despite the use of a carefully described standard method for the isolation of *Salmonella* from minced meat, the results from all laboratories were not the same. Differences between laboratories became especially apparent when samples were used with an even distribution of low numbers of *Salmonella* and high numbers of competitive flora. In this latter case four laboratories examined 100 artificially contaminated samples. The total number of samples found to be positive for *Salmonella* varied from 43 to 93. The differences were attributed to laboratory-associated factors such as differences in incubators, media preparation, time available to carry out the work, interruptions etc. When the analysis was carried out by the same workers, but in a single laboratory (repeatability), there were small (not significant) differences in the results. These differences were much smaller than when the analyses were carried out in different laboratories (reproducibility). The small differences were ascribed to the so-called 'human factor', that is interest, skill, dexterity etc.

Within the EC, existing and proposed Directives have implications for food and water microbiology laboratories with respect to standardisation methods and laboratory accreditation if there is to be mutual recognition of results within the single market. It is therefore important to be able to check both the performance of a method (national or international) as well as that of individual workers. The reference materials which have been and which will be developed and hopefully certified, will thus be available for the verification of both types of performance.

2.4. CONTROL CHARTS

When a fully validated method is available the analyst can envisage starting a statistical control system, including the follow up of the performance by control charts. The use of these charts obliges him to prepare a representative, homogeneous and stable reference material, which will be analysed at regular intervals. A control chart is a statistical device principally used for the study and control of repetitive processes. Shewhart originated control charts for the attainment of a state of statistical stability of a production process [35]. In fact these charts are simple graphs with in general a time scale on the horizontal axis and a quality characteristic such as sample mean, sample range or sample standard deviation plotted on the vertical axis. Shewhart suggested that the control chart might serve:

- firstly, to define the goal or standard for a process that the management might strive to attain;
- secondly, it may be used as an instrument for attaining the goal;
- thirdly, it may serve as a means of judging whether the goal has been reached.

It is thus an instrument to be used in specification, production, and inspection [35]. A standard book for an introduction to the quality control chart has been written by Duncan [36].

At the basis of the theory of control charts is a differentiation of the causes of variation in quality. Certain variations in the quality belong to the category of natural variation and are random. In addition, there may be variations produced by assigned causes, also called special causes or bias. Such assigned causes might be related to analytical problems in a particular series and their reasons should be investigated. It is the aim to take action in response to variation produced by special causes, but not to respond to chance variation. When the laboratory works at a constant level of high quality only few random errors persist and fluctuations in the results are small. Only then the method can be considered as fully validated and a statistical control system using control charts can be started. This will allow revealing the introduction of any *new* systematic error or drift and in some cases to monitor the precision when replicate measurements are performed. At regular intervals the analyst determines in a *reference material* the substance to be monitored and reports the result graphically.

2.4.1. Shewhart charts

To construct a Shewhart control chart the analyst must first prepare a representative reference material. The quantity must be sufficient to last for a long period of control, preferably as long as the measurements are performed. In a first stage the analyst applies his method several times on a test sample of the RM. A set of 15 measurements will allow him to calculate a mean reference value m with an associated standard deviation s . The measurements should be made over a prolonged period of time (e.g. separate calibrations, fresh setting of instruments etc.) so that a real reproducibility figure R represents s . If the method has been properly validated beforehand and is under statistical control, the analyst should be able to verify that the set of results is normally distributed. The values of m and s will then allow him to predict the range within which variations of measurement results will be acceptable. Figure 2.9 gives an example of a Shewhart chart. The 'Warning' limit corresponding to $\pm 2s$ tells the analyst that a possible problem occurred with the method but no immediate action is required. The 'Action, control or alarm' limit of the $\pm 3s$ value obliges the analyst to stop all measurements and to investigate for sources of drift. Statistics predict that 95.45% and 99.70% of data fall within the areas enclosed respectively in the ± 2 and $3s$ areas. The probability that a result falls outside one of these two limits is only about 0.55% and 0.30%. An unacceptable fluctuation occurs when a result falls outside the control limit or when twice successive results lie over the alarm limit (probability of 0.1%). Similarly, if the result of the measurement falls 11 times on the same side of the central mean line.

2.4.2. Other control charts

Beside Shewhart charts, also called X-charts, the analyst may apply R-charts (Figure 2.10) where the difference of two replicate measurements is plotted. R-charts give an indication of the repeatability of the method. X charts only warn for drastic changes

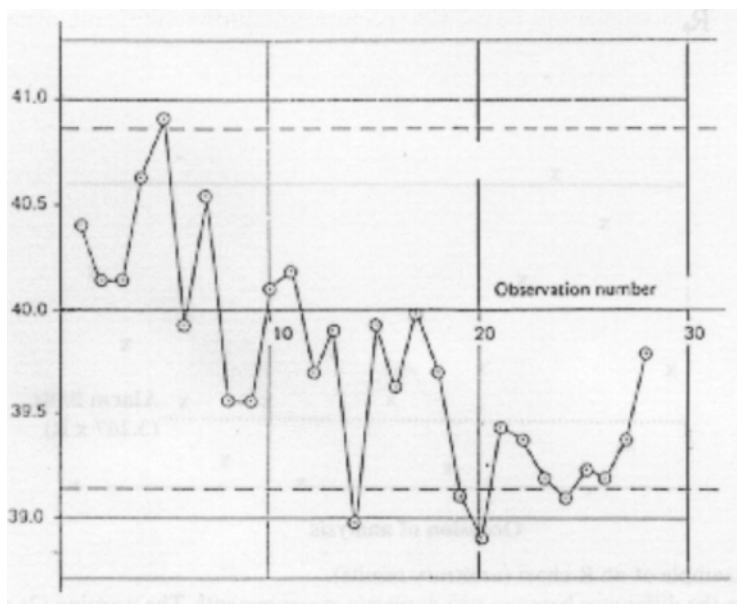


Fig. 2.9. Shewhart chart (the dotted line represents the $2s$ warning limits) of the determination of SiO_2 in water.

After observation day 10 a trend towards low results seems to appear. The Shewhart chart demonstrates only at day 20 that the measurements must be stopped (more than 9 times under the mean and 2 results under the $2s$ lower limit). The same data have been used to construct the Cusum chart of Fig. 2.11.

in methods or after several tests slow drifts are selected. Slow drifts in analytical procedures e.g. slow changes in the system caused by the ageing of parts of instruments, de-calibration in wavelength, ageing of calibration stock solutions etc., may be detected earlier by applying a Cusum chart (cumulative sum). Figure 2.11 shows an example of such a chart with the representation of the V-mask for the detection of the drift. In Cusum charts the analyst reports the cumulative sum of the differences of his determination with a reference value. If this value is linked to a CRM or by using directly a CRM, the Cusum chart allows us to monitor the accuracy of the measurement.

2.4.3. Control charts in microbiology

In microbiology two fundamental types of measurements are used by the analyst. The simplest ones consist in counting colonies on culture media in a Petri dish. Another principle consists in evaluating the most probable number of microbes by inoculating sub-samples into multiple tubes. The result of the latter is given by statistical tables. For both types of methods results are only available after a few days. For the presence of very few microbes, so-called presence/absence tests have been developed by microbiologists. They are mainly used for the detection of pathogenic microbes. For the last two types

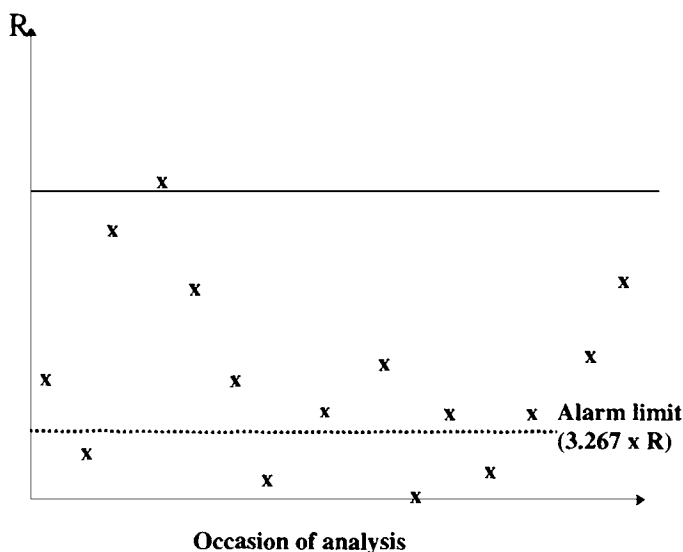


Fig. 2.10. Example of an R chart (arbitrary results).

R represents the difference between two duplicate measurements. The warning ($2s$ dotted line) and the alarm limit ($3s$ full line) are obtained from duplicate sets of results in a similar way as for X-charts.

of methods no control charts have been developed up to now. Havelaar et al. have developed together with adequate reference materials adapted principles of control charts for counts of large number of colony-forming particles [4,6]. As they are rarely given in practical terms in the literature they will be described in some detail. For additional information the reader should refer to the recent work of P. in't Veld [37] and of J.A. van Dommelen [38] from which the present information has been extracted.

2.4.3.1. High level counts

It has been shown that microbiologists can easily construct Shewhart type charts even when relatively low contamination levels are monitored. Figure 2.12 gives an example of a control chart of microbiology measurements. As for chemical measurements, stable and homogeneous reference materials are needed for constructing the chart. The use of control charts with averages as the quality characteristic is useful for controlling the average of the process, i.e. the average number of cfp in a standard volume. The statistics used in combination with these control charts are described in the ISO 8258 standard [39]. However, these statistics assume a Normal distribution of the data, which is not the case for microbiological RMs [40]. Heisterkamp and co-workers [41] showed, both on a theoretical and an experimental basis, that a logarithmic transformation of the microbiological counts resulted in data that behave approximately normally. The analytical results are therefore \log_{10} transformed before

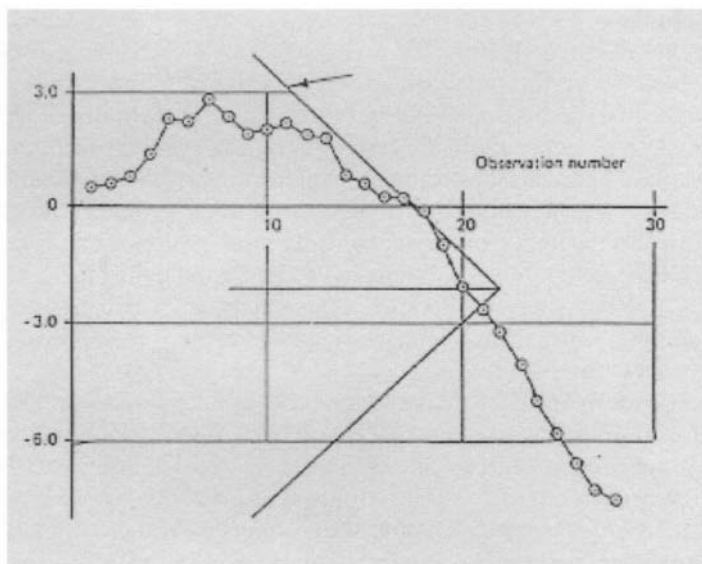


Fig. 2.11. Cusum chart.

Obtained with the same values as in Fig. 2.9. The sum of the differences between the found and a reference value are plotted in time: $Y_i = \sum_{j=1}^i (X_j - X_{ref})$. Cusum charts are used for the early detection of a bias newly introduced in the method. The V-mask technique is a graphical method which allows a rapid detection of drifts and trends. The angle of the mask and the distance between the angle-end and the observation day are arbitrarily chosen by the analyst (usually 45° and 2 days). The analyst is alerted when the cusum line touches the V-mask limits. In the example this happens at day, 3 occasions before the warning of the x-chart. For more stringent conditions the analyst can fix the angle at 30° .

the mean and standard deviation (s.d.) is calculated. For calculation of the s.d. the following formula is used.

$$s.d. = 0.8865 \cdot \bar{R} \text{ and } \bar{R} = \frac{1}{I-1} \sum_{i=2}^I x_i - x_{i-1}$$

where: s.d. = standard deviation.

\bar{R} = average moving range.

I = total number of capsules examined.

x_i = \log_{10} transformed count of the I^{th} capsule.

The s.d. is calculated on the basis of the difference between two succeeding \log_{10} transformed counts. This method of calculating the s.d. is preferred when only one sample is examined for each observation on the graph [39]. It will result in a more robust estimate of the s.d. compared to the usual way of calculating the s.d. It also means that this estimate of the s.d. is likely to be less affected by variations in counts due to assigned (systematic) errors [38] and results in more robust control limits. From the mean and standard deviation the following control chart limits are calculated on the logarithmic scale:

- warning limits : $\bar{x} \pm 2 \cdot s.d.$
- action limits : $\bar{x} \pm 3 \cdot s.d.$

After calculation of the mean and the control chart limits these values are back-transformed to the original. As a result of this transformation, single counts obtained from an RM can be plotted directly on the control chart without calculation of the \log_{10} value. The back-transformation will lead to an asymmetrical distance for the upper and lower warning and control limits around the geometric mean level. The geometric mean and the upper and lower control limits are drawn on the control chart as horizontal lines (see Figure 2.12). Each time an RM is examined the count is plotted on the graph, using the X-axis as the order of examination. The back-transformation of the mean and the control charts limits is not essential but is merely used to facilitate the notation of new observations.

The geometric mean and the upper and the lower control limits are calculated from the results of examination of the first 20 RMs. Each RM is examined once. The 20 measurements are preferably done on different days and by different technicians to cover within-laboratory variations. The counts of these RMs should be plotted on a separate graph, in order to check whether these counts meet the criteria stated below (i.e. that the analytical process is under control). If the result of one or more of these 20 counts does not meet the criteria, the cause(s) for this should be identified and a decision made about the validity of the count(s). If the cause can be identified (assignable cause) then this count should be disregarded and the geometric mean and control limits recalculated from the remaining counts. If the cause cannot be found then the

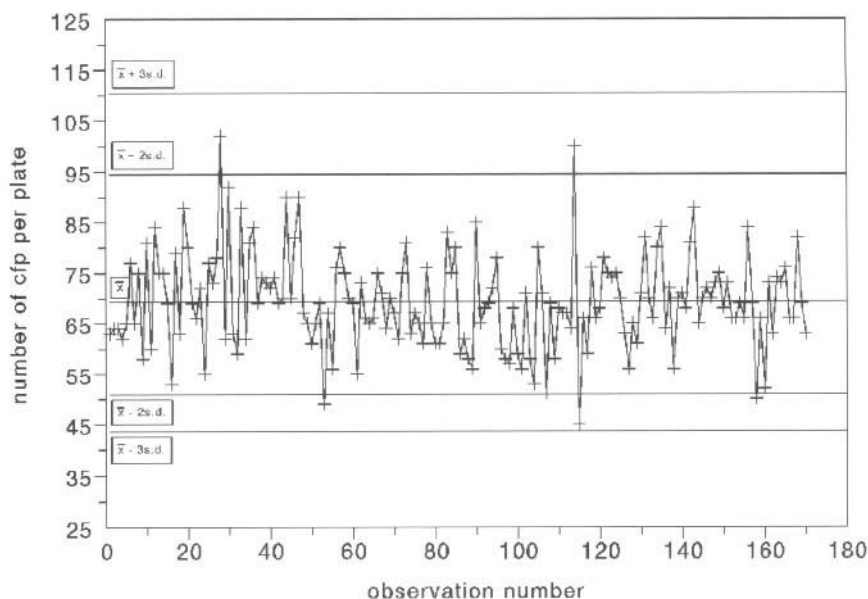


Fig. 2.12. Shewhart control chart using data obtained with the *B. cereus* RM (courtesy of P. in't Veld ref. [52]). The use of back-transformed data to draw the chart leads to asymmetrical upper and lower warning and control limits.

count should be regarded as belonging to the normal variation of the process and should therefore be included in the calculation of the geometric mean and control limits.

When the measurement process is under control, that is when any variation in counts is basically random, the counts obtained with the RMs, in 95% of the cases, fall between the lower and upper warning limits and in 99.7% of cases between the lower and upper action limits. When the variation in the counts does not conform to the pattern that might reasonably be produced by chance variation, then it is concluded that the process is out of control. It means that one or more systematic errors have been introduced into the system. Several tests for detecting out of control situations have been developed. The purpose of each test is to detect a particular non-random pattern in the points plotted on the control chart. These tests have been evaluated by Nelson [42,43]. The following criteria are used for interpreting the *microbiological control charts* [38,42]:

- (1) There is a single violation of the action limit ($\bar{x} \pm 3 \cdot s.d.$).
- (2) two out of three consecutive observations exceed the same warning limit ($\bar{x} \pm 2 \cdot s.d.$).
- (3) there are nine observations in a row on the same side of the mean.
- (4) six consecutive observations are steadily increasing or decreasing.

When a measurement process is under control, the chance of incorrectly obtaining a false signal that the process is out of control is less than 0.5% for each of the tests mentioned above. The overall probability of obtaining a false signal from one or more of these tests is *ca.* 1%. However, in practice these tests should be considered as simple practical rules for action rather than tests with specific associated probabilities [43].

When a measurement process is out of control, the cause(s) should be identified and a decision made on the validity of the count(s). The various tests are sensitive to different kinds of special cause of error in the measurement process and can be of help in identifying the cause(s). Nelson [43] gave an overview of each test and their sensitivity for different sources of error. Test (1) is sensitive to a single aberration in the process such as a mistake in calculation, an error in measurement, malfunctions of equipment and so on. Test (2) is sensitive to a shift in the process average although it is also somewhat sensitive to an increase in variation. Test (3) is sensitive to a shift in the process average and test (4) is intended to identify a trend or drift. No simple rules can be defined for judging the validity of results. This will depend on many factors, which are not always strictly related to the measurement process itself, e.g. the availability of replicate samples or the consequences of using the incorrect results.

In general, as for chemical measurements, Shewhart control charts are more sensitive for detecting incidental changes in the measurement process than for detecting small shifts in the average value of the process (drifts). To overcome such problems, the microbiologist can also use a Cusum chart [44]. To be able to judge whether or not a process is out of control a so-called V-mask is used. The most convenient form of V-mask is the truncated V-mask. The V-mask consists of three lines, one vertical and two sloping lines, called decision lines. The length of the vertical line is 10 times s.d. and the middle of this line is placed on top of the observation of interest, normally the last observation. The decision lines start at both ends of the vertical line and have a slope of $\pm (0.5 \text{ s.d.})$ per observation interval. Using this V-mask it is possible to judge whether or not a previous observation falls outside the mask, which indicates an out of control situation. An example of a Cusum chart with truncated V-mask is presented

in Figure 2.13. The data are the same as that used in Figure 2.12 for the Shewhart control chart. The average of the first 20 (\log_{10} transformed) observations was used for the calculation of the reference value. The same observations were also used to calculate the s.d. Another type of Cusum chart is described by Van den Berg and co-workers [45]. This type of chart consists of two charts, one for the positive deviations (S^+ chart) and one for the negative deviations (S^- chart). Each chart has its own reference value (k^+ and k^- for the S^+ and S^- charts respectively) that are 0.8 s.d. units above (for the S^+ chart) and below (for the S^- chart) the reference value. Negative Cusum values are plotted as zero on the S^+ chart and the reverse for the S^- chart. Each chart has an action limit defined as the reference value plus or minus (3.2 s.d.). Once the action limit has been exceeded the corresponding value in the S^+ or S^- chart is reset to zero. An example of such a chart using the same \log_{10} transformed data as for the other Cusum chart is presented in Figure 2.14.

Neither Shewhart nor Cusum charts can be used when the material examined is not fully stable. The stability of an RM is difficult to achieve and may not be obtained for all types of micro-organism. For those less stable materials alternative control charts are needed that take into account a change in the level of contamination [46].

2.4.3.2. Presence-absence reference materials

Control charts for processes in which the data are not Normally distributed are also possible. In the ISO 8258 standard [39] examples of control charts are presented that are based on binomial and Poisson distributions. These distributions could also apply

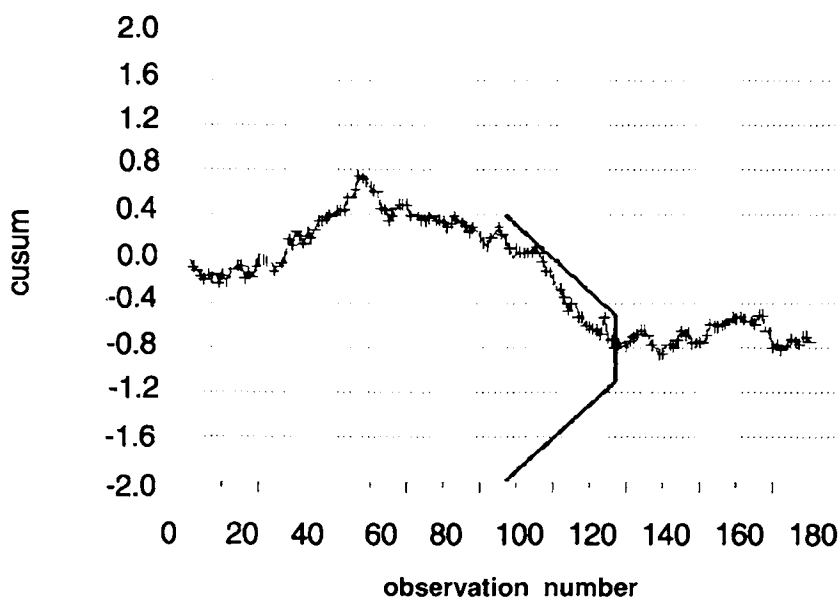


Fig. 2.13. Cusum chart with truncated V-mask. This chart has been constructed with the same data as those used for the Shewhart chart of Fig. 2.12 (courtesy of P. in't Veld [ref. 52]).

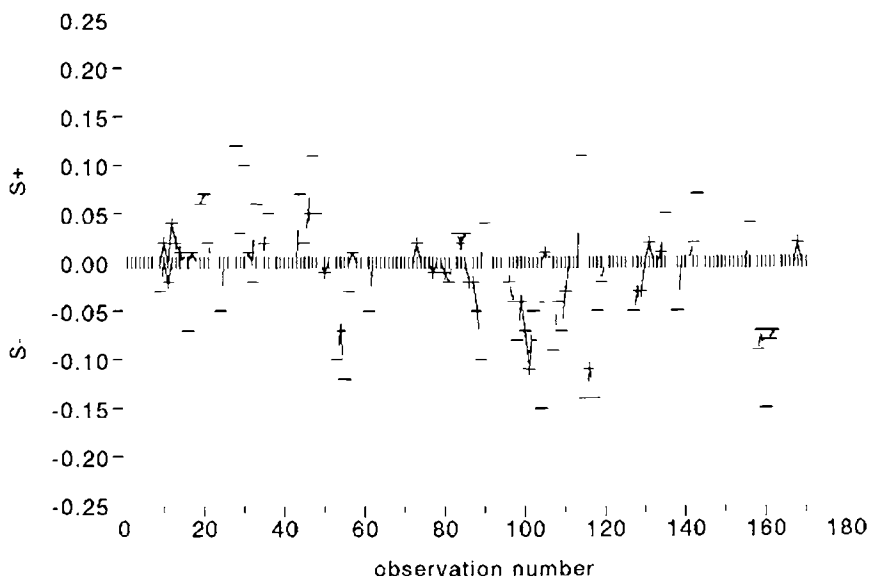


Fig. 2.14. Combined S^+ and S^- Cusum charts (courtesy of P. in't Veld [ref. 52]). This type of chart consists of two charts, one for the positive deviations (S^+ chart) and one for the negative deviations (S^- chart). Each chart has its own reference value (k^+ and k^- for the S^+ and S^- charts respectively) that are 0.8 s.d. units above (for the S^+ chart) and below (for the S^- chart) the reference value. Negative Cusum values are plotted as zero on the S^+ chart and the reverse for the S^- chart. Each chart has an action limit defined as the reference value plus or minus ($3.2 \cdot \text{s.d.}$). Once the action limit has been exceeded the corresponding value in the S^+ or S^- chart is reset to zero.

to results obtained using the presence-absence RMs. However, many replicates have to be made to obtain a single observation for such control charts. This will not be possible in practice using the milk powder capsule RMs produced by RIVM-SVM. Alternatives have been proposed by laboratories using these capsules and are discussed in detail by in't Veld [37].

2.5. WHERE AND WHEN TO USE RMS AND CRMS

As already mentioned above, at several stages of the analytical work and the control of its quality, reference materials (RMs) and certified reference materials (CRMs) are necessary and helpful tools. The present section will summarise all the steps in the work where and when they have to be used. Within the quality assurance scheme and at the three check levels RMs and CRMs are necessary. They are in fact essential for establishing the reliability of the analytical method, the operator and the laboratory as a whole. The practical use of CRMs for method validation will be discussed in Chapter 5 of this book. The general place of RMs and CRMs in QA/QC is shown in Figure 2.15.

Before going into the details of the use of reference materials it must be defined what RMs and CRMs are and which type of materials exist.

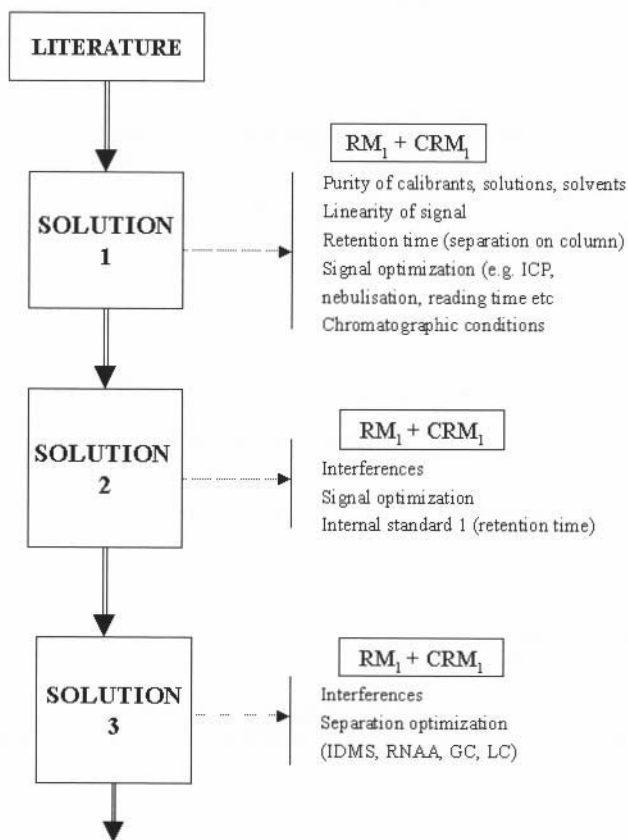


Fig. 2.15. Use of RMs and CRMs in the development and validation procedure of relative (Type II) methods for the determination of traces of substances in a solid matrix material.

For liquid or gas materials or for Type I methods some of these steps may be irrelevant. In addition all necessary transfer standards must be used to calibrate basic instruments (e.g. balances, wavelength etc).

RM1 and CRM1: pure substances or standard solutions (single substance or mixtures)

RM2: homemade clean extract spiked or not with RM1 (or CRM1)

RM3: homemade raw extract spiked or not with RM1 (or CRM1)

RM4: real solid sample spiked or not with RM1 (or CRM1)

CRM2: representative solid matrix CRM for the validation of accuracy

RM5: reference material for the continuous statistical control (control charts)

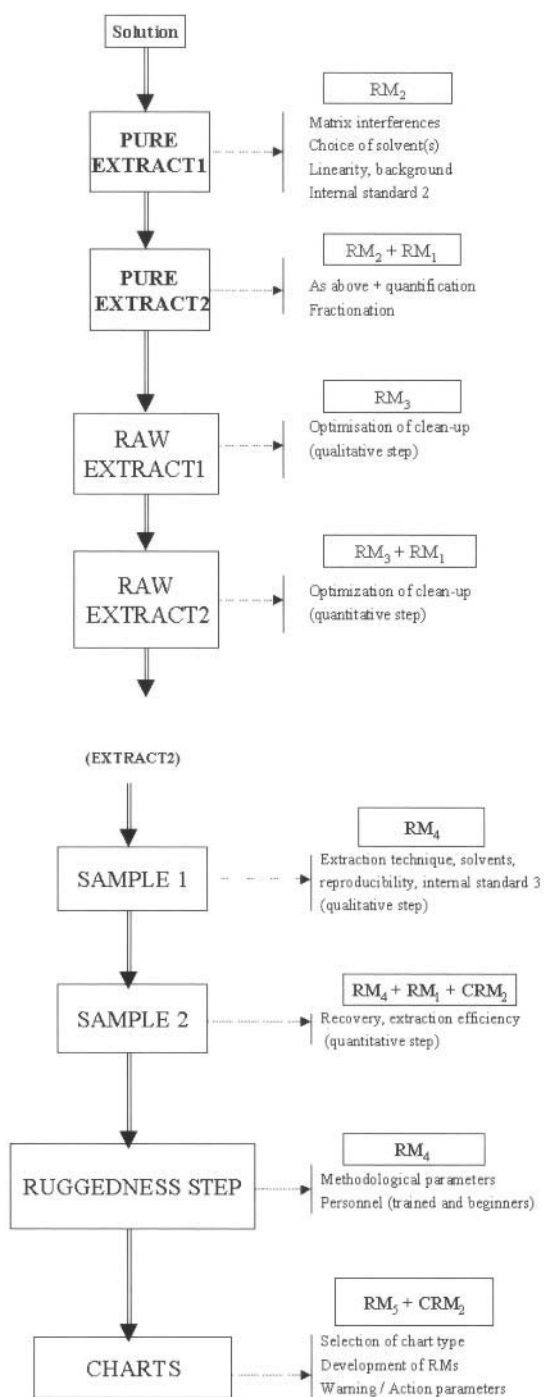


Fig. 2.15. Continued

2.5.1. Definition by ISO

Reference Material (RM): *A material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials [47]*

Certified Reference Material (CRM): *A reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realisation of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence [47].*

In fact the added value of the CRM lies in the existence of a guaranteed value of a property with its associated uncertainty. To achieve this added value, enormous analytical and in general scientific investments are required.

Primary Reference Material PRM: *a reference material certified with (a) primary method(s).* In practice such materials are mainly pure substances, manufactured materials, rarely matrix materials as already explained above. PRMs of matrix materials are very limited in number. NIST has available some CRMs certified with thermal ionisation-isotope dilution mass spectrometry (TI-IDMS) which has been classified by the Comité Consultatif sur la Quantité de Matière (CCQM) of BIPM as primary method. PRMs also exist for gas analysis. The Netherlands Meet Instituut (NMI) in Delft, The Netherlands has available several primary gas RMs prepared by gravimetry.

Figures 2.16 a and b summarise where RMs and CRMs can be used in the case of type III and type II methods. Two main categories exist: materials certified or not for the calibration of the instruments; and materials for the validation of the analytical method; RMs for the precision and development and control of the method, CRMs for establishing the accuracy.

2.5.2. Calibration and traceability

All analysts know that the majority of actual questions to be solved require the determination of traces of substances in sometimes tiny samples. They may be posed by industry for the development or the production of modern products showing a maximum added value, or to solve safety, health, justice or environmental problems. Classical, real chemical methods, are rarely adapted (e.g. titrimetry, gravimetry, etc.). Modern techniques of final determination are based on physical properties of the matter, e.g. spectrometry, where the measurement of the signal must be correlated with the concentration of the substance of interest in the unknown sample. Such methods using the effect of electrical or optical signals also allow automation of methods and consequently large-scale measurement strategies, sometimes with tremendous sample throughput. Up to several thousands of samples in clinical studies of new drugs, water or gas monitoring, can be run within a few days on several measurement devices. Calibration of such methods relies on the availability of pure primary substances, e.g. a chemical element, a chemical form of an element (e.g., metal speciation) or a chemical compound in organic or biochemical analysis. The correlation is established by means

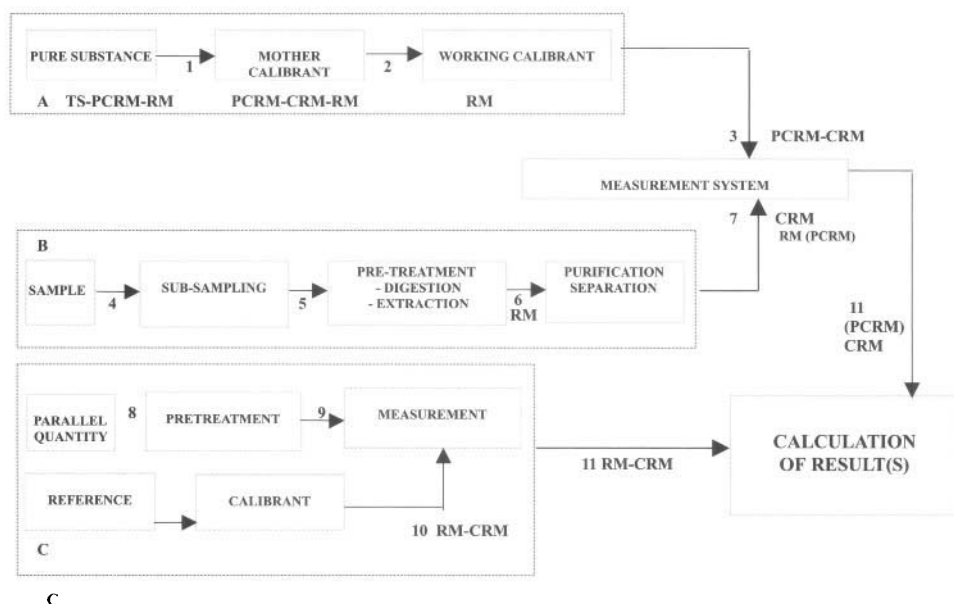


Fig. 2.16a. Steps in the analytical procedure of a relative or type II method (definition in section 2.1.3).

A: calibration chain; B: sample treatment, C: associated quantities. The numbers indicate the link between steps. TS: transfer standard (masses, wavelength calibration devices, etc), RM: laboratory or any non certified reference material, CRM: certified RM, PCR-M: primary CRM e.g. pure substances or matrix CRMs certified with a primary method (balance, IDMS etc). RMs and CRMs can be used at various stages of the measurement process. For chain A PCR-Ms or CRMs do not exist for all substances in particular for organic and organo-metallic determinations. For step 10, 11 and 12, PCR-Ms or even CRMs rarely exist. For step 6 laboratories may prepare raw extracts to follow the purification stage.

of a calibration curve. The solutions used to construct this calibration curve must be prepared with chemicals of an appropriate purity and verified stoichiometry [18,19].

Such calibration materials can be obtained under various forms and quality. When certified they are often considered as Primary Reference Materials (PRMs). As they allow one to establish the traceability of measurements to the fundamental units (mole, kg) of the S.I., they are used for the calibration step itself but also in the validation procedure when spiking of material is necessary.

Pure substances: e.g. metals, oxides, salts, pure organic compounds; they can be available with only stated purity figures or a certified purity. Certified purity values with a stated uncertainty are established on the basis of the measurement of impurities and exist mainly for metals and stable oxides, gases, sometimes salts, rarely for organic trace analysis (e.g. dioxins, furans, PCB, PAH from BCR or NIST remain exceptions). IRMM and IAEA also provide isotopic PRMs. This category also contains strains of organisms with fully identified genome e.g. bacteria, viruses, cells, parasites etc. used in microbiology, medicine, or agriculture (GMO identification). Pure substances are necessary for the calibration of relative methods (section 2.1.3).

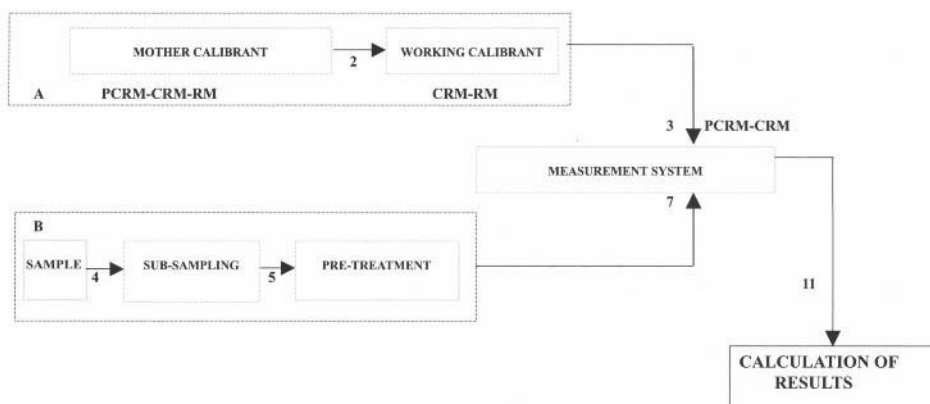


Fig. 2.16b. Steps in the analytical procedure of a comparative or type III method (definition in section 2.1.3).

A: calibration chain; B: sample treatment. The numbers indicate the link between steps. TS: transfer standard (masses, wavelength calibration devices, etc), RM: laboratory or any non certified reference material, CRM: certified RM, PCRM: primary CRMs e.g. pure substances or matrix CRMs certified with a primary method (balance, IDMS etc). For comparative methods chain C and several steps in chain A and B do not exist. It is shown in Fig. 2.16b by the absence of some step numbers which exist in Fig. 2.16a. RMs and CRMs can be used at various stages of the measurement process. For chain A) PCRMs or CRMs do not exist for all substances or materials.

Solutions or mixtures of one or more substances: mainly for trace element analysis, more rarely for organic traces, but often for alloys and gases. If they are certified on the basis of primary methods, e.g. gravimetry, and do not present risk of degradation they can also be called PRMs. Sets of materials of mixtures of pure substances are used for WDXRF calibration and in general for calibration of comparative methods (see section 2.1.3)

The use of the materials and the best chemical forms to be chosen have been discussed briefly in the section on validation. Details taken from Moody et al. and Wells are given in Tables 2.5a and b. The chemometric tools to be applied for the calibration of the signal and the calculation of the confidence interval of the calibration curve have been extensively detailed in several manuals and in particular the second book of Massart et al. [7] or in the text book of the FECS [48]. Modern automated spectrometers usually include adapted and properly validated calibration software.

2.5.3. Method validation

As shown in section 2.3 above, the method development and validation necessitate the preparation of several working materials to be able to verify each step of the method. Raw or cleaned-up extracts, spiked or not, spiked natural or artificial materials are all tools to which the operator will refer. In the method validation they serve mainly to establish the uncertainty of individual steps but also to verify the absence of losses of substance (spiked materials) or contamination (spiked materials and blanks). They are

TABLE 2.5A

EXAMPLES OF RECOMMENDED CALIBRATION SUBSTANCES AND FORMS WITH PRECAUTIONS FOR USE AND STORAGE — ORGANIC SUBSTANCES BASED ON REF. [23]

Family of substance (*)	Form of primary substance (PS)	Purity (certified or stated)	Mother calibrant (MC)	Working calibrant (WC)	Storage/use precautions
OCP	Crystals (CRM or RM) (1b)	> 99% majority	Concentrated solution (CRM or RM) single or mixture (1a,b)	Diluted solution (hexane, toluene, iso-octane etc) RM (2)	Sensitive to UV and temperature; sealed ampoules for MC (3) (4)
Polar pesticides (5)	Crystals (rare CRMs, RM)	> 98% majority	Concentrated solutions (RM)	Diluted solutions	Often highly unstable in solution, change often MC and WC
PCB	As above OCP (1b,c)	> 99% (1c)	As above OCP (1a,b,c)	As above OCP (2)	As above OCP (3) (4)
PAH	As above OCP (1c)	> 99.5% (1c)	As above OCP (1a,b)	As above OCP (2)	Many PAH are unstable replace MC and WC often (3) (4)
PCDD/F	As above OCP but purity not always reliable	> 98% (1c)	Concentrated solutions (1b) or mixtures (1c)	Diluted solution with labelled congeners	Stable but very diluted solutions, risk of contamination
CB/CP	Crystals (RM mainly)	Available > 98%	As above OCP (no CRM)	As above OCP (no CRM)	Some CP unstable
Phenols	As above CP/CB	Available > 98%	As above CP/CB	As above CP/CB	As above CP
Surfactants	Pure substances	Often doubtful	No CRM	No CRM	Purity rarely guaranteed
Natural toxins	Rare, Crystals (no CRM)	Unknown	No CRM	No CRM	Often natural origin

(*): for abbreviations see glossary

(1) a: EPA mixtures b: NIST certified crystals or mixtures c: BCR certified crystals or mixtures

(2) Rarely CRM available as concentration must be adapted

(3) MC: weighing before and after use indicates if losses happened, storage in refrigerator

(4) WC: weighing before use as for MC, can be stored in ampoules or tight vials, storage in refrigerator, avoid serial dilutions, use mass basis to dilute and calibrated balances.

(5) Covers several families of substances e.g. phenyl-urea, organo-P, -N, etc.

TABLE 2.5B

EXAMPLES OF RECOMMENDED CALIBRATION SUBSTANCES AND FORMS WITH PRECAUTIONS FOR USE AND STORAGE — INORGANIC SUBSTANCES BASED ON REF. [22]

Group in periodic table	Elements and form (1)	Purity of substance	Commercial solutions	Remarks
Group I (2)	Li (Cl) (CO ²⁻ ₃) Na, K (Cl) Rb, Cs (Cl)	> 99.9% > 99.9% > 99.9%	SRM and RM (3) SRM and RM (4) SRM and RM (4)	Some unreliable solutions on market, carbonates, oxides, chlorides and nitrates: doubts on stoichiometry
Group II	Be, Mg (metal)	> 99.9 (Be) and 99.999% (Mg)	SRM and RM (4)	As above Group I
Group II	Ca, Sr, Ba (CO ²⁻ ₃)	Up to 99.999%	SRM and RM (4)	As above Group I
Transition 1st row	All metals (Sc also as oxide)	Sc > 99.9% all others > 99.99%	SRM and RM	As above Group I
Transition 2nd row	All metals (Y also as oxide)	Nb > 99.9%, all others > 99.99%	SRM and RM	As above Group I
Transition 3rd row	All metals except (NH ₄) ₂ OsCl ₆	Os > 99.9%, Au, Hg > 99.9999% Others > 99.999%	SRM and RM	As above Group I
Lanthanides	Oxides, metals highly reactive	> 99.9% some up to 99.999%	SRM and RM (4)	Stoichiometry of oxides not always sure
Actinides	U metal, Th oxide	> 99.99%	SRM and RM	Isotopic CRM are available from nuclear material suppliers (5)
Group III	B: oxide Al, Ga, In, Tl: metals	> 99.99% > 99.999%	SRM and RM (3) SRM and RM (4)	As above for lanthanides
Group IV	C: graphite, formic acid, ethanol, sucrose	Graphite > 99.999% others > 99.99%	RM	Graphite used for spark source MS, others for C determinations

TABLE 2.5B

CONTINUED

Group in periodic table	Elements and form (1)	Purity of substance	Commercial solutions	Remarks
Group IV	Si, Ge, Sn, Pb metals	> 99.999%	SRM and RM (4)	Si to be handled with care, Pb shows isotopic variability
Group V	N: gas	> 99.9999%	SRM and RM	Nitrate salts and P oxides are not easy weighing forms (hygroscopic)
	N: NH_4Cl	> 99.9%		
	P: oxides	> 99.999%		
	P: phosphoric acid	> 99.99%		
Group V	As, Sb, Bi metals	> 99.99%	SRM and RM	As Group I above
Group VI	O: water	> 99.999999%	SRM and RM	Correction for density or temperature
	O: gas	> 99.99%		
Group VI	S elemental	> 99.9999%	SRM and RM	Stoichiometry (water content) must be verified
	sulfuric acid	> 99.99%		
Group VI	Se, Te metals	> 99.999%	SRM and RM (4)	As Group I above
Group VII	F, Cl: sodium salt	> 99.99%	SRM and RM	As group I above
Group VII	I: sublimed, K salt	> 99.99%	SRM and RM (6)	As Group I above
	Br: K salt, Br_2	> 99.99%		

(1): only the preferable choice is given, others also exist, sometimes in very pure forms (> 99.99%)

(2): pure metals not easy to handle because too reactive towards water and air.

(3): may suffer isotopic variations.

(4): RM solutions often available as nitrates or chlorides

(5): New Brunswick Laboratory (Argonne, USA), EC-JRC-IRMM (Geel, Belgium)

(6): SRM from NIST in USA

Quality in chemical and biological analysis

used at the validation stage but can also be used later on to allow the statistical control system the detection of the introduction of a systematic error in the method and when revalidation of some steps becomes necessary. If complex, difficult or very expensive materials are concerned (e.g. dioxins, toxins) the analyst may wish to conserve the materials prepared for the validation of the method for further use. In this case the materials prepared for the validation must be stable enough and properly stored to remain reliable. CRMs for the validation of the accuracy of intermediate steps of methods rarely exist. BCR once produced a fly ash extract for the validation of the chromatographic separation and the purification of PCDD and PCDF determinations but this material was withdrawn from the market for stability reasons [49].

2.5.4. Statistical control

Setting up statistical control tools such as control charts necessitates the preparation of proper reference materials by the analyst. Their preparation follows the same basic requirements as CRMs: they must be homogeneous and stable over time to allow comparability of results. They do not require traceability or accuracy to be demonstrated and engage only the responsibility of the analyst who produced them to himself and his laboratory. They are used at regular intervals as can be seen from Figures 2.9 to 2.11. The frequency of use depends on many analytical parameters (robustness of method, number of samples, precision requests, etc.), regulatory or economic parameters (measurement frequency, size of batches, customer requests, etc.). Such working standards, also called laboratory reference materials (LRMs), may additionally be compared with similar CRMs. If such a link can be made it allows the accuracy of the method to be maintained within the statistical control systems. It must be noted that this traceability to CRMs is not easy to achieve in one single laboratory and that it would be preferable to have the LRMs analysed by different good laboratories, which apply different methods, as is often done by certification bodies. For simpler determinations in known matrices, e.g. elements in drinking water, a CRM can be introduced from time to time within the control chart as shown in Figure 2.17. This can also be achieved for more complex matrices when a CRM similar to the LRM exists.

For regulatory measurements of goods and products (e.g. agricultural products like grain, soils, fertilisers) or trade related product control measurements, laboratories can find non certified RMs on the market of very good quality and at prices affordable for daily control. These producers often provide services for proficiency testing of laboratories.

2.5.5. Microbiology

As described above, microbiological measurements are of a different nature than chemical measurements. Nevertheless, the needs for quality control are similar. RMs and CRMs are only available from a few suppliers and their use is linked to the type of material delivered. Capsules of RMs available from SVM in the Netherlands and lenticules from PHLS propose the same type of approach. These materials are added to the matrix — water or food — before the measurement procedure starts. BCR-CRMs

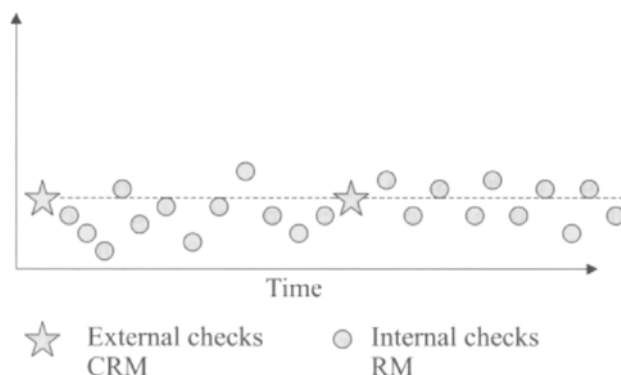


Figure 2.17. CRMs can be introduced into the statistical control scheme provided they are similar to the RM used or that a known stable relation exists between RMs and CRMs. This is mainly the case with simple or perfectly known matrices (e.g. drinking water, gases).

are gelatine capsules similar to the RMs of SVM but have a content of bacteria certified through a given standardised procedure.

High count RMs and CRMs: Mainly used for indicator organisms for water analysis and foreseen for total spoilage flora for food, they allow one to count large numbers of cfp. The incidental use of high count CRMs is detailed in the chapter on CRMs. The analyst has basically to optimise the number of capsules or lenticules to be used. This number depends on the difference that needs to be detected between a true laboratory geometric mean and the certified value of the CRM, or an assigned value to a RM. The difference that can be detected is strongly dependent on the stability and homogeneity and on the variance of the assigned value. This already explains the difference between certified and non certified materials as CRMs are delivered with smaller variances. Both types of RMs (capsules and lenticules) can be used for method validation, medium control and control charts as described above, and in interlaboratory performance studies. They have the advantage of being much more stable than real contaminated samples (e.g. water) and can therefore be used over longer periods and in very large scale studies. The European Commission has supported the development of proficiency testing schemes for water testing laboratories over several countries (Germany, Italy, Spain, Ireland, Greece, Portugal, Belgium) involving more than 300 participants [50]. All of them received the same material in the same week. Only robust and stable materials such as the lenticules produced by PHLS and the capsules of SVM could assume such large-scale distributions. Real water samples could not be produced and shipped to so many laboratories.

Low count materials: RMs and CRMs with low contamination levels (low level materials) are used to verify the performance of presence/absence methods. They are analysed to detect small differences between a laboratory fraction of negatives and the certified or assigned value. In contrast with high level RMs and CRMs, it makes sense to analyse more capsules in parallel because then there is additional benefit in the ability to detect (smaller) differences.

2.5.6. Conclusions

In many cases and circumstances of the daily quality control of analytical work RMs and CRMs are helpful tools. Very often RMs are sufficient, in particular for statistical control actions. Where a rough estimate of accuracy or even precision is sufficient, a simple RM or calibration material is also largely adequate. However, for the establishment of the accuracy in the procedure of method development and validation, for revalidation of modified methods or whenever the analyst needs to demonstrate accuracy, e.g. measurements for court cases, CRMs should be employed as they have the advantage of being certified. It will be up to the operator and the laboratory's quality management to determine when, where, and how RM or preferably CRMs shall be used.

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Chapter 3

Use of Certified Reference Materials

3.1. GENERAL ASPECTS

Before going into the details of their production, it is useful to know what one can expect from CRMs and where and how to practically use them. For all those who have been active over years in the field of CRM development and production, it is always astonishing how little is known of the existence and the advantage of using CRMs. This starts at the analyst level because there are only very few comprehensive and widely accessible publications on the subject. Bodies like EURACHEM, CITAC and ISO-REMCO have taken over the task to produce user-friendly information, but they have only very limited means. The European Commission has sponsored several events in this field but they have only reached a limited targeted public [1]. Finally, it must be recognised that the existing ISO Guide 33 [2] on the use of CRMs — also called ‘proper use and misuse of CRMs’ — is difficult to understand and poorly illustrated with real chemical measurement cases (matrix CRMs). This Guide summarises the main objectives that a CRM can fulfil. They have:

a structural role for:

- transferring and maintaining knowledge
- helping the analyst to achieve accuracy/trueness and consequently traceability to recognised references
- linking measurement results with the S.I.

a practical role for:

- helping to assess the measurement process.

3.1.1. Structural role of CRMs in measurement sciences

The first objective and role of a CRM is the crystallisation of analytical knowledge into a material and the maintenance of this knowledge in time through its availability to the analytical community. In the section on CRM production the implications in terms of preparation and verification of the reference material will be discussed. Development of CRMs often requires preliminary studies involving material as well as analytical disciplines. Only when a material is properly certified and when all the work done for its production is well described does the material achieve its information role. Each user will benefit from this developed knowledge. By making this CRM available to the analytical community and by using the newest analytical methods in the process of certification or re-certification of a new batch, knowledge is kept up to date. As CRMs are available to all analysts, each set of material assures the transfer of

the knowledge much better than any scientific publication would do. This transfer and availability of knowledge is ensured in space, e.g. NIST, NRCC, NIES, BCR etc., materials are available worldwide without commercial restrictions, and in time, e.g. some BCR materials have been in the catalogue since the early 1970s and some NIST materials were certified at the beginning of the 1960s. Many others are regularly replaced.

CRMs, at least those which can claim that they are traceable to a recognised reference or standard, e.g. the S.I. unit, can be used to link the measurement to this reference. Such CRMs can be compared to transfer standards, as they are known and used in physics, e.g. mass transfer standards. Unfortunately, except for pure primary substances, such materials hardly exist in chemical measurements for complex materials. Pure materials are often the only real link to the basic S.I. unit of amount of substance, the mole, for many measurement processes. They intervene in fact mainly in the calibration process (see Figure 2.17). Finally, some CRMs, e.g. those used to test material properties or activities (e.g. pH, conductivity, etc.) can be used to realise measurement scales for these properties. The last two structural roles in measurement sciences also represent the primary practical role of CRMs as they intervene directly in the measurement process.

3.1.2. Practical role of CRMs

The analyst may be more interested in the practical role and use of CRMs. This role lies mainly in the calibration of instruments and signals and in the validation of a measurement process. Depending on the objective of the measurement, the analyst will adapt the analytical method and consequently the validation process and the role of the CRMs. Applying a method to establish the acute contamination of river sediments by pesticides released from a chemical plant does not necessarily require the use of a CRM certified for the relevant pesticide. Monitoring the slow disappearance over years of the pesticide from the same sediment actually requires a method of the highest precision and trueness. Here, a well-adapted CRM would mean a material with a similar matrix to the studied sediment and showing a certified pesticide content with a small confidence interval. In that case, the accuracy of the applied own method can be reliably estimated. The choice and the use of the CRMs are linked to the type of method and the final objective. Calibration materials and validation matrix materials are both of primary importance. Unfortunately, suppliers rarely provide the user with relevant information on the use of the CRMs that they sell. Many CRMs are only available with their certificate on which are stated the certified value, the confidence interval of this value, and limited information on practical use (e.g. sample intake). Major suppliers indicate the analytical methods used for the certification. Sometimes more detailed reports are referenced in the certificate. Only BCR systematically provides the user with a certification report that gives extensive indication on the way the certification was achieved and all the relevant values used to calculate the certified value. In fact, this reflects the origin and fundamental objectives of BCR, which were mainly to improve the quality of measurements performed in the European Community: a more pedagogic approach, rather than simply producing quality control tools.

When ISO Guide 33 refers to the instructions for use of the CRMs that should be given by the supplier, it must be admitted that this is often non-existing information.

Much has still to be done in the field of 'proper use and misuse of CRMs'. In this chapter, we will try to translate into understandable analytical language how to use CRMs and particularly matrix CRMs for method validation. It must be stressed that the subject is only rarely discussed in the literature. Therefore, examples will illustrate the principles. Finally, we will also give some advice on the use of CRMs in microbiology.

3.2. TYPES OF CRMS

CRMs can be:

- (a) pure substances, or solutions to be used for calibration and/or identification;
- (b) materials of a known matrix composition for the calibration of a certain type of measuring instrument, e.g. spark source emission spectrometry, X-ray fluorescence (XRF), and those techniques which require a calibration with a material similar to the matrix analysed;
- (c) matrix reference materials which, as far as possible, represent the matrix being analysed by the user and which have a certified content (such materials are mainly used for the validation of a measurement process);
- (d) methodologically-defined reference materials which are dependent of the measurement method used: leachable or aqua-regia-soluble fractions of trace elements from soils, ashes and slag; bioavailable fraction of a certain element, extractable pesticides, etc.). The certified value is defined by the applied method following a very strict analytical protocol.

In practice, CRMs are of the same use as RMs. The main advantage of using a CRM lies in the availability of the certified value, which is a key to achieve trueness. As the CRMs are certified with fully validated methods, following strict quality control procedures and within selected laboratories, the uncertainty of the certified value is also a key element for the user.

3.2.1. Calibration materials

Following the classification of the analytical methods given by ISO 32 [3] two major type of calibration materials can be certified. For relative methods such as all spectrometric ones, pure substances are necessary. They can be certified for the stoichiometry and degree of purity but also for isotopic composition. The latter case is a prerequisite for measurements of radioactive materials and for stable isotope mass spectrometry (isotope dilution TIMS or ICP-MS). For comparative methods, pure substances and mixtures of substances are necessary, as well as matrix materials for which the element to be determined is perfectly known and also the major compounds that produce a matrix influence on the signal (e.g. alloys, gases).

3.2.1.1. Pure substances

Table 3.1 resumes the situation in terms of primary reference materials for instrument calibration. Several levels of quality exist depending on the analytical field.

TABLE 3.1

RELATION BETWEEN TYPE OF CALIBRATION AND TYPE OF METHOD

Type of method	Calibration mode	Calibrant	Examples of methods	Degree of uncertainty
Type I or calculable method	Direct through fundamental laws (1)	Mass of reagent (volume) towards mass of sample	Titrimetry Coulometry Gravimetry	Small uncertainty limited to mass determination and purity of reagent and reaction product
Type II or relative method	Calibration curve	Set of calibration samples towards (set of) sample	All spectrometries (2)	Large due to complex methods, matching of sample and calibration sets
Type III or comparative method	Calibration curve	Samples and calibrants of similar composition	WDXRF, automatic analysers of water, coal, etc.	Very large as calibration samples are measured with (Type I or) Type II methods

(1) Mass action law, Nernst relation, etc

(2) Inorganic: atomic absorption, -emission, -fluorescence, X-ray fluorescence, mass spectrometry; organic: chromatography with MS, ECD, FID, UV, fluorescence etc.

3.2.1.1.1. Element and ion determinations

The essential aspects have been discussed in the introduction on the use of RMs and CRMs. It should be noted that inorganic CRMs, in particular pure metals, are available on the market from several reliable suppliers. They show usually purity values with associated uncertainties that are negligible compared to the uncertainty of the majority of spectrometric methods in which they serve as calibrants. It is usual to find materials of stated (not by definition certified) purity of 99.999% (five nines in analytical jargon) or better. This would mean that any impurity is below 0.001% as a mass fraction. No relative analytical method has precision performances that go down to such levels. Suppliers of 'ultra pure' metals are numerous. NIST sells such metals as certified RMs (SRMs). The certification of the purity is discussed briefly in Chapter 5. It can be mentioned that the measurements are often based on absolute methods. The ultimate detection of impurities can be made with spark source MS. For pure metals the uncertainty linked to the calculated purity is small. Therefore, compared to the intended use and the uncertainty of classical methods applied by the analyst for the determination of elements, it is totally negligible.

When leaving the field of metals, many elements cannot be obtained as pure elements (A^0) but only in forms of salts or oxides. In this case, purity figures lose 'nines'. Those elements, which can be obtained in an oxide form, present usually at least one stable

oxidation stage. They can be stored in a way that they are not subject to alterations such as variations in water of crystallisation. Moody gives several examples of such substances [4]. Purity figures of 99.999% are still usual for oxides and consequently rarely affect the precision of the total measurement of many spectrometric systems. Only in TI-IDMS these purity figures may represent a significant uncertainty. Those concerned know how to tackle the problem of uncertainty calculation in their uncertainty budgets. For other inorganic substances such as salts (e.g. chlorides, sulphates, nitrates etc.) purity may become a significant limiting factor. In particular, storage of these substances becomes difficult as many of them are hygroscopic. Therefore, the stated purity may only be valid at the first use and may become questionable afterwards. But suppliers still offer materials of stated purity of 99.999% for many elements of the periodic table (see also table 2.5). Whenever the purity becomes a significant factor for the accuracy to be achieved, the operator has to correct its weighing result for the purity stated and eventually he must also take into account the identity of the major impurities. This is the case when multi-elemental determinations or multi-residue analysis of organic substances are performed.

Similar conclusions can be drawn for ionic determinations. Pure salts or elements can be obtained as well as highly pure reagents such as acids and bases, ions can be produced in a very reliable manner. It is another story when dealing with organic substances.

3.2.1.1.2. *Gases*

All common gases exist in very pure forms and can serve as calibration substances in routine measurement. It is, together with metal analysis, the best provided field in terms of primary reference materials. One important producer is NMI, who has developed several procedures to produce gases traceable to the mole of substance. NIST has also a large offer in pure gases. All commercially important industrial gases are nowadays available. Toxic gaseous substances are not yet all available.

3.2.1.1.3. *Organic and organo-metallic determinations*

With the substances of organic composition the analyst enters a more difficult world. Only very few organic substances have been certified so far for their purity. The methodologies to achieve such a certification are far from established. NIST and BCR have on their respective catalogues several substances available in solid form (crystals) or in solution. EPA has accredited several producers of certified organic substances; they have produced several substances for environmental monitoring, in particular pesticides. Polycyclic aromatic hydrocarbons (PAH), some polychlorobiphenyls (PCB), phenols, chlorophenols, chlorobenzenes etc., are available. BCR has produced several sets of certified calibration solutions for dioxins and furans for monitoring incineration gases and ashes. These standards also contain the ^{13}C isomers. Purity figures for organic substances often range from 98% to 99.99% (BCR CRM pure PAH crystals). BCR has also recently certified an organic form of As, arsenobetaine, at a purity level of 99.7% [5,6]. Compared to the number of organic trace analyses performed every day, the number of pure certified substances is very limited. The quality of the proposed primary substances by commercial suppliers is sometimes very low. It has been noticed in several

BCR studies that substances on the market had purity figures of sometimes less than 70% (even 50%) where they were stated to be pure at 98%! This demonstrates that in organic and organo-metallic trace analysis impurity, and in general uncertainty due the quality of primary calibrants, cannot be neglected and are sources of important bias in measurements. Therefore, it is of importance for analytical chemists to identify reliable suppliers. If necessary, it may be necessary to purify purchased substances. Collaboration in such studies and exchange of substances is one of the most useful outcomes one can get from colleagues. BCR projects have often initiated such relations.

The determination of the purity of an organic or organo-metallic substance is difficult. The methods to identify and quantify impurities are limited and poorly sensitive compared to inorganic determinations. Stoichiometry might also be difficult to be assessed properly (hydrates, chlorohydrates, etc.). Impurities can be of inorganic or organic origin. They can be suspected or deduced from the synthesis route chosen; for natural substances extracted from plants or microbes (e.g. natural toxins) this does not apply. Organic impurities must be analysed in both liquid and gaseous separation systems. Elemental analysis, melting point determinations, thermo-gravimetry techniques etc. can all help, as well as ^1H NMR for the establishment of the identity. The uncertainties of the determinations of organic impurities remains usually high compared to inorganic substances. Therefore, one might be obliged to conservatively attribute to the purity a large uncertainty. When the purity is known or certified the analyst must correct for the presence of impurities. When the certified purity is accompanied by an uncertainty that is large, e.g. for organic substances, then he must include it as a systematic source of error into the uncertainty budget.

3.2.1.2. *Synthetic mixtures*

The synthetic mixtures of pure gases, metals or oxides are often necessary for the calibration of 'comparative methods' e.g. metals for WDXRF. They are available for nearly all industrial applications for ferrous and non-ferrous alloys. The industrial sector sometimes provides the market. Mixtures of oxides or salts also exist. Many sectors of industry applying rapid methods for production monitoring like the metal industry have their own sets of RMs. NIST provides many CRMs of this kind. In the less classical fields like nano-material technologies and bio-materials only few or no CRMs exist at all. This demand will be growing and must be filled in the near future; homogeneity problems are one of the major difficulties in the production of such materials. They concern only marginally the control of the environment. Restricted scientific fields such as archaeology have their own needs in terms of primary CRMs. For this purpose, BCR has developed a set of copper alloys mimicking ancient copper samples [7]. The manufacturing of such mixtures can be done with a very high level of precision and consequently fully negligible uncertainties on the final measurement, e.g. mixtures of gases. In many cases, the preparation requires techniques which induce losses of elements or may bring contamination (oxygen in metals). Therefore, a simple certification of such mixtures on a gravimetric basis is not always possible. In this case other methods must be used e.g. spectrometric methods and, consequently, the uncertainty on the content of the individual constituents is largely higher than the uncertainty due to the

purity of the single compounds and the weighing. In such cases the uncertainty may affect the calibration of the instrument which uses these calibration mixtures.

Mixtures of several elements or substances in solvents are available from many suppliers in certified quality. Such materials are very useful to environmental monitoring as many represent excellent materials for the calibration of instruments. Several target contaminants like PCB, PAH, PCDD and PCDF, metals etc. are available from NIST, BCR etc. Such certified materials have purity figures with small uncertainties, they can be used without any particular precaution compared to the equivalent pure substance. They are valuable tools to detect bias in calibration and allow rapid correction. Uriano and Gravatt [8] have cited an example of the use of CRMs to correct for bias in SO₂ measurements in air and in particular how CRMs can help to correct for additive or multiplicative bias in interlaboratory studies. Massart et al. have also discussed similar effects on signals of detectors [9] and the reader should refer to them.

3.2.1.3. Natural materials

For the calibration of comparative methods and in particular WDXRF, but also for automatic analysers and other spectrometric methods, many natural materials have been developed for several years. This concerns mainly the analysis of ores, cements, raw materials, etc. These materials are certified on the basis of spectrometric methods and, as for some synthetic mixtures, the calibration will be affected by the uncertainty of the calibrant.

3.2.1.4. When using certified calibrants

Certified pure substances and mixtures are used whenever it is necessary to assess the reliability of a calibration performed with non-certified materials. Where the market does not supply sufficiently reliable working standards e.g. organic, organo-metallic substances, toxins, etc. it might be useful to prepare mother solutions of calibrants only with CRMs. Then, the working standards are dilutions made regularly from these stock solutions. A quality control procedure of the mother solutions must be installed, e.g. weighing of the stored vials before and after sub-sampling to detect evaporation of solvent, examination of chromatograms of concentrated solutions to verify the absence of degradation products or relative peak ratios between substances. For metals and in general many inorganic substances, the market supplies good quality materials. In such cases, CRMs are less or even not necessary once a reliable supply is identified (many of these suppliers trace their materials back to producers of CRMs). The use of CRMs in such analysis may be restricted to regulatory or forensic measurements where a certificate for the calibrant is requested to avoid problems in court. For many comparative measurements e.g. WDXRF, it is preferable to use CRMs only, if they exist. Far more important and interesting in the field of environmental monitoring are matrix materials. They fill a gap in the method development, validation and control process more than anywhere else, and can be considered as method validation materials.

3.2.2. Method validation materials

CRMs to finalise the method development, to validate analytical procedures and finally control in time the accuracy of procedures, are rare and valuable materials, in particular matrix CRMs. They should tell the analyst how his entire measurement procedure is performing. He will receive information on precision as well as on trueness. CRMs are primarily developed to check for trueness, which is the most difficult property to verify. Precision can be tested on RMs or can be estimated from published data e.g. the performance required by a standard method, whereas the evaluation of trueness is possible only with external help: a CRM or a properly organised interlaboratory study. Having a CRM allows one to perform the verification of trueness whenever the operator wants it. The analyst should never forget that only when accurate results (precise and true) are achieved, comparability in space and over time is guaranteed. But to exploit to a maximum the information on trueness delivered by the CRM, the precision must also be sufficient and verified.

3.2.2.1. Choice of the CRM

The criteria for the choice of the CRM are not different from the criteria to select the material for the preparation of a laboratory reference material for method development, statistical control charts etc. The difference lies in the availability of adequate CRMs from reliable suppliers and the level of compromise which the analyst must make between an ideal situation and the reality of what is on offer. Massart and co-workers have proposed a principle component analysis to help select the best adapted CRMs available on the market to verify AAS analysis of foodstuffs [10]. Their approach took into account the analytes as well as the matrix composition. Besides the fact that they highlighted a lack of sorts of CRM, in particular those having a fatty matrix, they demonstrated that such a statistical approach can help in the most appropriate selection of materials. Boenke also proposed a systematic approach for the choice of materials to be certified for mycotoxins [11] and which could be followed by potential users. The selection of the CRM by the analyst should include a certain number of parameters; this can cover the following properties to fulfil the intended purpose:

- level of concentration of the analytes;
- degree of homogeneity to allow the use of small sample size (important for forensic investigations mainly);
- matrix similar to the real samples: chemical composition but also physico-chemical binding of analytes;
- a form similar to real samples so that identical pretreatments can be performed (particle size and distribution, etc.)
- quantity sufficient to test the method properly;
- stable enough to be stored easily without having a risk of alteration of the CRM;
- having property values: certified value and uncertainty compatible with performance criteria expected by the user; therefore, the certification procedure must be adequate.

Many of the above properties will be discussed in the chapter dealing with the production of CRMs. The performance criteria required from the CRMs and the way to exploit them are discussed here.

3.2.2.2. *Validation of the precision of a method*

A true method without precision (whatever this could really mean!) does not tell us anything. Before verifying trueness, the analyst must assess the level of precision he has achieved. The study of the reproducibility R and repeatability r of the method is the preliminary task of the validation procedure and can be largely done with own laboratory RMs. However, in order to know if the achieved performance of R and r are comparable to the actual state of the art of the method or of the market, the analyst can use a CRM. When later on he tries to assess the trueness of his method he must also achieve a precision performance which allows the CRM to tell him about his trueness performance. ISO Guide 33 [2] helps to evaluate simply the precision performance criteria. The use of BCR-CRMs, which are certified by interlaboratory studies where several independent and different methods are used, can help a bit further.

3.2.2.2.1. *Within laboratory following ISO 33*

The revised version of this guide gives an approach for the use of CRMs. After having validated his method (see Chapter 2) with intralaboratory tools, the analyst analyses the CRM. He calculates the mean value \bar{X} of at least five determinations, possibly ten. These determinations have to be fully independent. This means that he starts five or more times from a fresh test sample and does a new calibration of the instrument. If the same operator does the job, in a short period of time, it can be considered as having been done under repeatability conditions (r). Otherwise a reproducibility value is established (R). Having calculated the mean \bar{X} and the standard deviation s ,

$$\bar{X} = \sum_{i=1}^n X_i / n \quad s = \sqrt{\sum_{i=1}^n (X_i - \bar{X})^2 / (n - 1)}$$

where X_i represents each individual result of a total of n independent measurement results (at least five, preferably more). Outliers should not happen anymore at this stage, unless an 'accident' occurred during a measurement. In case doubts remain, a Grubbs test will clarify the situation. With the mean and standard deviation, ISO Guide 33 explains how to use the CRM parameters to assess the precision of the method.

The use of the CRM, the information it bears and the quality of the material it represents, tells the operator that his s value is due to the measurement precision of his method. If relevant, the CRM should tell him which parts of the uncertainty of the measurements are not due to the analytical method but could come from inhomogeneity of the material. Unless very small sample intakes of solid materials are used (solid sampling AAS, INAA), this inhomogeneity should be negligible. At least in using CRMs for environmental monitoring, if the analyst compares his value of s with a stated,

required repeatability or reproducibility figure σ , he can estimate his own performance in terms of precision. The comparison is made by a χ^2 test. The value of σ can be found in different ways.

Value of σ : the required precision value σ can be found in the certificate of the CRM itself: σ can be the uncertainty of the certified value when the same method as the certification method is used by the analyst. It can also come from the individual set of measurement values of one of the methods used in the interlaboratory certification study. In such a case, all individual data and the methods must be available from the certificate or the certification report. Such information is given in some CRM reports of BCR and is illustrated in Annex 3.1. The σ can also be stated in a written standard as a minimal or target precision value to be obtained. Finally, σ can also simply come from the laboratory itself which applied another method previously or with another instrument or from another laboratory experienced with the method, or it may be requested by a customer. Care must be taken in extrapolating σ simply from another element or substance which is analysed in parallel using a multi-elemental or multi-residue methods, as these may not be comparable at all because of matrix effects etc.

The ISO Guide 33 does not indicate how to establish or find σ . The χ^2 test is performed as follows:

$$\chi^2_c = (s/\sigma)^2 \quad (\text{calculated } \chi^2_c) \text{ and}$$

$$\chi^2_{table} = \chi^2_{n-1, 0.95} \quad \text{are compared:}$$

$$n-1$$

if $\chi^2_c \leq \chi^2_{table}$ there is no reason that the method is not precise enough

if $\chi^2_c > \chi^2_{table}$ there is a reason to suspect that the precision is not sufficient.

χ^2_{table} values can be found in the tables given in all statistical textbooks. If the precision is sufficient, it is worthwhile to verify the degree of trueness of the method.

3.2.2.2. Precision through interlaboratory study using ISO 33

The precision of a method can also be assessed through an interlaboratory study. This gives a much better estimate of the real precision the method and the laboratory can achieve. The way to properly organise such studies is described in Chapter 12.

Performance of the method in one laboratory: The assessment of the *precision achieved with the method in one laboratory*, still compared to the *a priori* performance of the method σ , but within an interlaboratory study is similar to the approach used above with the within-laboratory precision. With the consideration of the number p of sets of data delivered by the participants:

$$\chi^2_{table} = \chi^2_{p(n-1), 0.95} \quad \text{and} \quad \chi^2_c (s/\sigma)^2 \text{ are compared:}$$

$$p(n-1)$$

if $\chi^2_c \leq \chi^2_{table}$ there is no reason that the within-laboratory precision is insufficient

if $\chi^2_c > \chi^2_{table}$ there is a reason to suspect that the within-laboratory precision is insufficient.

Performance of the method between laboratories: When the real *precision performance of the method* has to be established it must be done through an interlaboratory study. The reproducibility between laboratories is the real precision one can expect from the method. It is evaluated on the basis of the following statistics (p laboratories performing n replicates):

S_m : long term precision of the method in a single laboratory (or if unknown, uncertainty of the certified value of the CRM)

S_b : the between laboratory standard deviation in the interlaboratory study

S : within laboratory standard deviation as above

σ : as above the expected precision

$$\chi^2_c = (s^2 + ns_b^2) / (\sigma^2 + ns_m^2)$$

In fact, χ^2_c can be reduced in many cases to ns_b^2/ns_m^2 because the within-laboratory deviation s and σ are negligible towards the between-laboratory differences.

$$\chi^2_{table} = \chi^2_{p-1, 0.95} \quad \text{and} \quad \chi^2_c \text{ are compared:}$$

$$p-1$$

if $\chi^2_c \leq \chi^2_{table}$ there is no reason that the between-laboratory precision is insufficient

if $\chi^2_c > \chi^2_{table}$ there is a reason to suspect that the between-laboratory precision is insufficient.

In all cases the laboratory or the group of laboratories which test the method benefit from the high quality of the material (homogeneity, stability, representativeness as described in Chapter 4 on production of CRMs) but also may benefit from the uncertainty of the certified value. Such a test should be done for all standardised methods before they are accepted and published.

3.2.2.2.3. Precision using BCR Certification reports

BCR certification reports give much more information than the usual certificates issued by producers of CRMs. In particular, BCR gives all individual results obtained in the certification campaign. BCR-CRMs are the result of preliminary feasibility studies and improvement schemes where methods were validated in detail through successive interlaboratory studies (see Chapter 12) before certification is launched. Therefore, it has always been the policy to also give details to the user on how to achieve results usable for certification with different methods. The certification based on different independent laboratories applying different fully validated methods allows collecting various sets of data. The user of the CRM can exploit this information when using the CRM to assess the precision of his own method.

Assessment of precision with a similar method: When a material is certified with different methods, the precision performance of the methods may be very different.

The certifying body uses these different methods to detect a possible systematic bias of a single method. But the user might not be satisfied to test his precision performance with the overall precision performance calculated from all these methods. When applying INAA for Cd, the user might wish to exclude from the precision evaluation data issued from AAS which is much less reproducible. Similar situations might exist when applying isotope dilution mass spectrometry in organic trace analysis of PCBs compared to GC-ECD or when using GC-MS for PAHs compared to HPLC-UV (see Table 3.2 on PAH and Cd). Having all individual data available in the certification report, the user of the CRM may recalculate the precision performance as above but based on the data issued by the method(s) of interest only. The values of s_m and s_b must be recalculated by the user.

3.2.2.3. Validation of the trueness of a method

As already mentioned, the main advantage of CRMs lies in the availability of the 'true value'. In other words, it is a reference value that is considered as the best estimate analytical sciences can give for the real content of the substance in the particular material. As such CRMs represent the only way to check trueness easily. The verification of trueness will consist in a statistical comparison of the value determined by the operator on the CRM and the certified value. Again it must be stressed that this has no sense if the method's precision is too large. The trueness of a method can be verified within the laboratory or through an interlaboratory study, in particular when a reference standard or an official method is concerned.

3.2.2.3.1. Within laboratory assessment of trueness

After having verified the precision of the method (n replicates), the laboratory uses \bar{X} and s (as defined above) and compares them to μ the certified value of the CRM. The uncertainty of the certified value should be negligible towards the real reproducibility figure of the measurement method s_m .

The comparison is done as follows:

$$-a_2 - 2s_m \leq \bar{X} - \mu \leq a_1 + 2s_m$$

TABLE 3.2

EXAMPLES OF PRECISION FIGURES (CV OF FIVE MEASUREMENTS)
OBTAINED FOR INAA AND AAS IN SOME CRMS FOR Zn

Matrix	INAA	AAS
Rye grass (CRM 281)	6.8%	10.9%
River sediment (CRM 277)	5.2%	2.5%
Lake sediment (CRM 280)	1.2%	2.6%
Mussel tissue (CRM 278)	7.0%	2.2%
Cod muscle (CRM 422)	3.7%	1.4%

If the difference between the found mean value and certified value is between $+ \text{ or } - 2s_m$ and an added factor, the method delivers true results. ISO Guide 33 introduces here adjustment factors a_1 and a_2 . These two factors represent an acceptance of a systematic deviation or bias towards low or towards higher values. The factors a_1 and a_2 may be different. They are based on technical, regulatory or commercial stipulations. Let us consider a measurement process for a regulated or commercial purpose e.g. the content of an element in ore or an alloy. The customer will accept the ore only if the content of the element of interest is higher than a certain amount. To protect himself he will not accept a method showing a systematic bias towards high values. He may set a_1 to zero. He may accept too low values from the method, as he does not lose money if he accepts the ore, but still needs to know the content of the element to adjust the price. He may set a_2 at a certain level of acceptance differing from zero. Because of reasons of rapidity for delivering results he may also accept that the methods presents a certain bias towards low and high values: less accurate but more rapid. Such commercial stipulations are not common in environmental monitoring, but safety aspects may oblige the setting of one sided limits of bias to methods. Technical reasons may also exist e.g. sensitivity of the method, which oblige the analyst to accept a certain flexibility towards lower values.

3.2.2.3.2. *Between laboratory assessment of trueness*

The assessment of trueness of a method using an interlaboratory study is possible and desirable to evaluate the real trueness performance of the method. The approach is similar to the within-laboratory trueness assessment but the mean value \bar{X} is obtained from the overall mean of individual results. s_D is the standard deviation of the overall mean of the interlaboratory study with p sets of n data and the s_D is given by:

$$s_D^2 = U_m^2 + s^2/n$$

p

The comparison is done as above:

$$-a_2 - 2s_D \leq \bar{X} - \mu \leq a_1 + 2s_D$$

The stipulation factors have the same meaning as above. Such an approach is mainly used for reference methods.

3.2.2.3.3. *Use of BCR certification reports*

When having all individual data available from the certification report, the analyst may wish to have a closer insight into his performance in terms of comparison to the results achieved by a similar method used in the certification. In the case of materials certified for organic trace substances e.g. dioxins in fly ashes, where methods inevitably remain affected by small systematic errors, the uncertainty calculated from the set of data of the interlaboratory certification study may appear small compared to the spread of the accepted sets of data. The half-width of the 95% confidence interval of the set of data is used as uncertainty. The user can adapt the way of determining the value of s_D above. He may wish to replace the 95% confidence interval by a tolerance interval

or just by the range of values accepted for certification. Such approaches are not specified by ISO Guide 33. Therefore, such changes may be hardly accepted by third parties when the CRM is used to provide a demonstration of the trueness of the method. This is a field of investigation that needs serious consideration by CRM producers, IUPAC and ISO-REMCO. It is of primary importance for all those involved in trace analysis, in particular organic traces in environmental or food samples. It has a practical implication for a laboratory. The example in Annex 3.2 is taken from a certification report, and illustrates various ways of testing trueness. It refers to the determination of 1,2,3,6,7,8-HxCDD in BCR-CRM 607.

3.2.3. Frequency of use of CRMs for method precision and trueness

How often should a laboratory use a CRM to verify that the precision and the trueness of its method is still under control? There are in fact no absolute rules. It will depend on the number of analyses performed, the robustness of the method, economic reasons and regulatory demands.

Frequency: This covers two aspects: the number of measurements per run or set e.g. capacity of the machine with automated sampling systems, the number and frequency of the sets e.g. every day or regrouped on certain days with longer periods where the instrument is used for other purposes. Keeping the system under statistical control implies that it is followed by control charts. These charts will in fact realise the verification of the reproducibility in time. A CRM used inbetween will allow one to assess that accuracy is also maintained. This is possible when the laboratory RM used for the control chart and the corresponding CRMs have been thoroughly compared beforehand. In such cases CRMs may be used from several times every week to once or twice per year, depending on the robustness of the method and the internal quality assurance system of the laboratory. Analyses which lead to the construction of charts are by definition performed on a routine basis with high throughputs e.g. water monitoring, sediment analysis, plants, waste water and sludge release, exhaust gas and particle analysis in stacks etc. For many of these measurements CRMs are available. For measurements, which are performed only on rare occasions, or with instruments, operators and supplies also engaged in other tasks, less quality control systems exist (e.g. no control charts). In such cases it might be interesting to revalidate the method for precision and trueness with a CRM at each occasion of measurement.

Changes in the measurement system: When important changes occur in a routine measurement system, the procedure must be revalidated and it is wise to finish this revalidation by using a CRM. Such changes can be due to the involvement of a new operator, a change of instrument (spectrometer, incubator for microbiology, extraction system, software or peak integration system etc.) or a new batch of consumables which has been established to be a sensitive part of the method (e.g. new calibration material, new extraction solvents, new batch of culture medium for microbiology etc.). These changes may be rare but also incidental.

External reasons: For particular demands of customers or because of severe implications of the measurement results on commercial agreements or judicial decisions, the laboratory may wish to back-up the measurement of the sample by a validation

measurement with a CRM. Second line controls (see the corresponding section) may also use CRMs, e.g. comparison of performance of operators, instruments, methods, by introducing them into the analytical system. The fact that they are often recognised by the analyst because of their aspect (homogeneity, dryness) and the limited sources of CRMs that allows us to identify their origin, limits this use.

3.3. VALIDATION OF STANDARDS AND REGULATORY METHODS

Matrix CRMs are also used to test a standardised method; the use of a standardised method is not a guarantee of accuracy in itself. Good standards help to avoid method development errors (e.g. gross systematic errors) but not implementation errors. Therefore, before using a standardised method in a laboratory for delivering results, the method must also be validated and in particular for the aspects of trueness. This may be restricted to a simple check of performance by using a CRM as stated above. For the validation of the standard itself an interlaboratory study with precision and trueness check on a CRM is necessary; this is described in sections 3.2.2.1. and 3.2.2.2. Unfortunately, standards with established performance criteria based on CRMs are still rare also because of the availability of adequate CRM. Many older written standards are restricted to a precise description of the method without any indication on expected performance nor any way of establishing the performance in the user's laboratory. Fortunately, standardisation bodies such ISO and CEN have recognised the need of accompanying their standards with performance criteria.

Matrix CRMs are of absolute necessity when the standard has the objective to determine a fraction of a substance, or when the method used affects or even defines the parameter measured e.g. microbiology. Such CRMs are increasingly developed in biological or environmental areas. Examples are given in this book. They are also encountered in the agricultural and food sector and in the biomedical field where they help to assess the comparability of calibration kits of instrument manufacturers.

3.4. COMPARISON OF METHODS AND INSTRUMENTS

Evaluation of the performance of a method or an instrument represents the largest use of CRMs. Examples are widely reported in the literature. One could even say that the development of a new method or instrument without evaluation of the performances with (a) CRM(s) is an incomplete task. Besides these research tasks, CRMs for calibration or validation are also used to assess the performance of instruments by the manufacturer himself to demonstrate the possibilities of his instrument or by the customer who wishes to evaluate the proposed instrument before purchasing it. CRMs produced by independent official or regulatory bodies to validate instrument performance or calibration sets have been under development for several years. They have in particular allowed the solution of inaccuracy problems in the biomedical sector where calibration test kits of automatic instrument manufacturers were not comparable and even led to different results between countries; such arguments supported many BCR projects for

the development of CRMs in the biomedical sector. Similar tasks can be performed using CRMs to compare national standard methods of analysis in various fields. BCR has solved several problems of this kind in the agriculture, food and feed fields. In such areas CRMs help to lift technical trade barriers and have helped to resolve conflicts between countries on environmental problems.

3.5. EVALUATION OF NON-CERTIFIED REFERENCE MATERIALS

When a laboratory has developed and validated fully its analytical method, in terms of precision and trueness, statistical control tools will allow maintaining the achieved performance and avoid drifts. As mentioned earlier, CRMs cannot fulfil statistical control tasks in laboratories. They are too expensive for such purposes and their supply is limited. CRMs also cannot fulfil all the needs in terms of organisation of interlaboratory studies (proficiency testing). Non-certified reference materials must be developed for such a purpose. In order to give information on trueness to the users these 'secondary RMs' can be linked to CRMs. As long as simple calibration materials are concerned or mixtures of pure substances, comparability can be achieved quite easily. When matrix materials are concerned, in particular solid phase matrices e.g. soils, tissues, seawater etc., difficulties of comparability appear. Together with differences in matrix composition, differences in the behaviour appear during the analytical procedure. Therefore, such secondary matrix materials are difficult to produce by a single laboratory and collaborative productions are often necessary. Direct traceability to 'primary matrix CRMs' is nearly impossible. The production of natural matrix CRMs requires a similar approach as the one used to produce certified RMs.

3.6. CRMS FOR MICROBIOLOGICAL METHODS

3.6.1. Types of microbiology CRMs

Despite the burning need for RMs and CRMs in microbiological measurement disciplines only very few materials exist. Basically, the type of materials, in terms of objectives, necessary for chemists are also needed by microbiologists.

Pure cultures of microbes also called banks of microbes: Fully genetically identified micro-organisms are stored and used as references for testing laboratories to characterise their 'homemade strains'. They could be compared to banks of pure chemical reference substances. These microbes are living organisms maintained in a few reference laboratories, which will realise the identification work of strains sent by testing laboratories.

(Certified) Reference materials of artificial composition: In this category fall the CRMs and RMs produced by BCR and RIVM (Rijksinstituut voor Volksgezondheid en Milieu in the Netherlands). They consist in gelatine capsules containing artificially contaminated milk powder. Others have been available for several years in interlaboratory performance studies e.g. lenticules from the Public Health Laboratory Service North in Newcastle (PHLS — UK), egg yolk etc.. Usually these (C)RMs are added to the

matrix on which the measurements are to be performed (water, meat, milk etc.). The RMs serve usually in interlaboratory studies, or as intralaboratory quality control samples e.g. for control charts. They could be considered as a kind of standard addition material.

Real matrix materials: They are rare because often unstable. They serve as external quality assessment materials (proficiency testing) in particular in the field of water monitoring. In food microbiology they are rare or unknown.

In all three categories, only the contaminated milk powders of BCR produced by RIVM have been certified. They still represent the only certified RM on the market.

3.6.2. Use of BCR-CRMs in microbiology

Two situations must be considered which lead to particular rules for using CRMs:

- counting of large numbers (more than 50) of colony-forming particles (cfp); such large numbers concern indicator organisms (e.g. faecal contamination) for monitoring surface water quality or spoilage flora in food;
- testing the presence or absence of bacteria or counting very low numbers of cfp (e.g. less than 5), e.g. the detection of pathogen organisms in water or food.

In all cases, the analyst will count cfp. The RMs of BCR are not certified through most probable number techniques (MPN). Microbiological CRMs are always certified through (a) given method(s) i.e. the certified value depends upon the analytical method. For all these reasons and particularities, special rules for the use of CRMs in microbiology have been developed by RIVM for BCR. The following sections dealing with the use of CRMs are directly extracted from a report prepared by J.A. van Dommelen [12].

3.6.3. CRMs with high levels of cfp

In the certificate of analysis of a CRM, tables with 95% confidence limits of the certified value are given in relation to the number of capsules and the number of replicates. The certificate does not state the optimal number of capsules or replicates a laboratory should use. An example of a certificate of *Bacillus cereus* (BCR-CRM 528) is given in Annex 3.3. Statistical methods can be used to determine the required number of capsules and replicates that allow a good judgement of an experiment and that is realisable in practice.

A true mean for the number of cfp, in one analytical portion, for the studied technique and for a particular laboratory, μ_{lab} is obtained through experiments. To determine μ_{lab} precisely, a very large number of capsules should be analysed. In practice, only a limited number of capsules can be analysed. With the results of this small number of capsules an estimate, called m_{lab} , of the mean number of capsules for the laboratory can be computed. This value of m_{lab} is an estimate of the unknown μ_{lab} .

When a laboratory wants to know if its true mean, μ_{lab} , is similar to the certified value, μ_{cert} , i.e. the number of cfp in one analytical portion, the analyst compares m_{lab} with μ_{cert} . If m_{lab} is within the 95% confidence limits (C.I.), as can be found in the tables for users in the certificate, the laboratory concludes that μ_{lab} is comparable to μ_{cert} . The

95% C.I. means that the probability to conclude that $\mu_{lab} = \mu_{cert}$ is indeed the case in 95% of the cases.

When m_{lab} is much larger or smaller than μ_{cert} , the conclusion will be that μ_{lab} is different from μ_{cert} . It is possible that a wrong conclusion about μ_{lab} is drawn from the results of the experiments (m_{lab}). When the conclusion that $\mu_{lab} \neq \mu_{cert}$ is drawn, but in reality $\mu_{lab} = \mu_{cert}$, a type I error occurs as shown in Table 3.3. m_{lab} is assumed representative for μ_{lab} , but in reality is not. The probability for a type I error is commonly called α i.e. the probability of concluding from the experiment that a significant difference exists between μ_{lab} and μ_{cert} . Type I errors should be limited as much as possible, α should be low e.g. for the certification of RM α was 0.05.

It is also possible that μ_{lab} is really different from μ_{cert} . Two possible conclusions can be drawn from the experiment (see Table 3.4).

It is possible that a wrong conclusion about μ_{lab} is drawn from the results of the experiments (m_{lab}). When m_{lab} is equal to or in the neighbourhood of μ_{cert} , the conclusion will be that $\mu_{lab} = \mu_{cert}$. When this conclusion is wrong, but in reality $\mu_{lab} \neq \mu_{cert}$, a type II error occurs (see Table 3.4). m_{lab} is assumed to be representative for μ_{lab} , but in reality is not. The probability for a type II error is commonly called β i.e. the probability of concluding from the experiment that no significant difference exists between μ_{lab} and μ_{cert} .

Type II errors should be avoided as often as possible. An appropriate choice of β is generally in the range of 0.05 to 0.20. Larger values of β are not really acceptable since the chance of missing a major difference becomes too high. Usually $\beta = 0.20$ has to be accepted because in practice most experiments have a limited size (number of tests) and the lower the value of β , the more capsules and replicates have to be analysed.

There is a fourth possible situation, namely where the experiment leads to the conclusion that $\mu_{lab} \neq \mu_{cert}$ (see Table 3.4). The probability for this situation is $1-\beta$ and is called the power of the experiment. The power is the probability that a difference, if present, is detected. A value of β of 0.20 leads to a power of 0.80. This section concentrates on a power of 0.80 ($\beta = 0.20$).

Both α and β , govern the way of using the BCR-CRM capsules, the number of

TABLE 3.3
USE OF HIGH LEVEL CONTAMINATED CERTIFIED REFERENCE MATERIALS IN MICROBIOLOGY. THEORY OF TESTING THE METHODS: TYPE I ERROR.
 α IS THE PROBABILITY OF CONCLUDING INCORRECTLY THAT THE RESULT OF THE TEST μ_{lab} DIFFERS SIGNIFICANTLY FROM THE CERTIFIED VALUE μ_{cert} .

		Conclusion based on the result of the experiment	
		$\mu_{lab} = \mu_{cert}$	$\mu_{lab} \neq \mu_{cert}$
Reality	$\mu_{lab} = \mu_{cert}$	Correct! 1 - α	Type I error α

TABLE 3.4

USE OF HIGH CONTAMINATION LEVEL CERTIFIED REFERENCE MATERIALS IN MICROBIOLOGY. THEORY OF TESTING THE METHODS: TYPE II ERROR AND POWER OF THE TEST.
 α IS THE PROBABILITY OF CONCLUDING INCORRECTLY THAT THE RESULT OF THE TEST μ_{lab} DIFFERS SIGNIFICANTLY FROM THE CERTIFIED VALUE μ_{cert} .
 β IS THE PROBABILITY THAT THE TYPE II ERROR OCCURS.

		Conclusion based on experiment	
Reality	$\mu_{lab} = \mu_{cert}$	$\mu_{lab} = \mu_{cert}$	$\mu_{lab} \neq \mu_{cert}$
		Correct! $1 - \alpha$	Type I error α
	$\mu_{lab} \neq \mu_{cert}$	Type II error β	Correct! $1 - \beta$
			↑ POWER

replicates and the degree of significance the analyst can draw from the results of his tests.

3.6.3.1. The number of capsules to be used

For microbiological CRMs with high levels of cfp, tests are based on the geometric mean. The required number of capsules and replicates will depend on the level of differences the analyst wants to detect between the certified value and the laboratory geometric mean and on α and β . Of course, any difference is relevant and must be detected but this is unrealistic since the number of capsules to be used for such a level of acceptance would be infinitely large [13]. It is economically also unrealistic.

Large differences between the certified value and a true laboratory geometric mean can be detected with a small number of capsules.

Example: use of *Bacillus cereus* (CRM 528) MEYP agar ISO 7932 method

The certified value according to the certificate of analysis shown in Annex 3.3 is 53.4 after 24 h incubation [14]. If the laboratory analyses one capsule in singular (i.e. without replication) a difference of 64% can be detected. This can be concluded from Figure 3.1, because the true laboratory geometric means are 32.5 and 87.6. The percentage of difference is computed from the lowest value, the certified value or the true laboratory geometric mean.

The power is minimally equal to $\alpha = 0.05$. When the true laboratory geometric mean is equal to the certified value the upper half of Table 3.4 applies and then the probability of concluding from the experiment that they are not equal is still α .

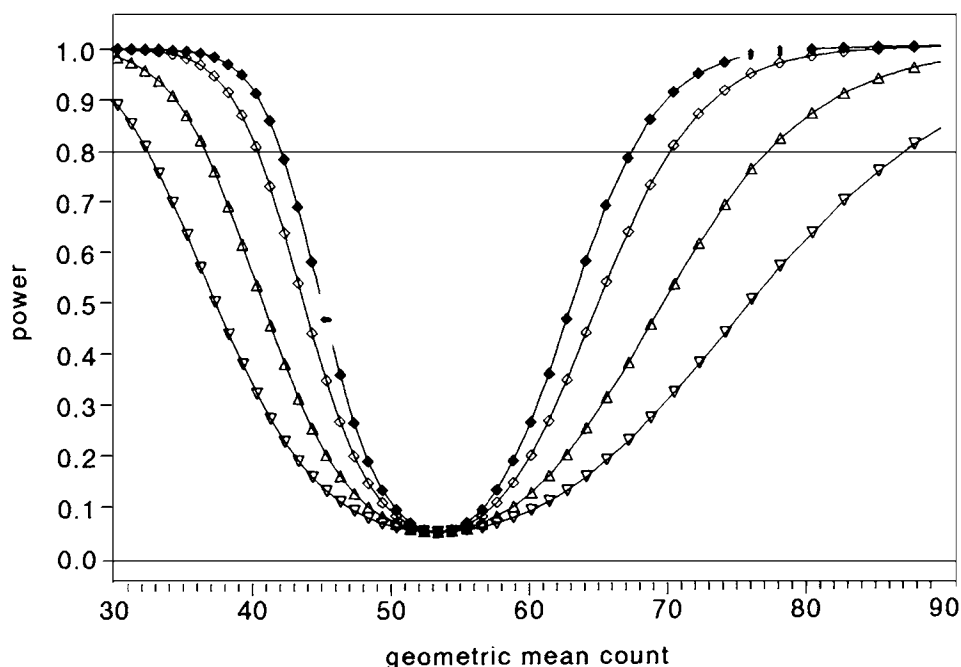


Fig. 3.1. Relationship between the power, the geometric mean and the number of replicates ($J = 1 \nabla$; $J = 2 \triangle$; $J = 3 \diamond$; $J = 4 \blacklozenge$) for the enumeration of one capsule of *Bacillus cereus* CRM (MEYP agar after 24 h incubation at 30°C, certified value 53.4).

When more capsules are analysed, smaller differences can be detected. Table 3.5 shows that when a laboratory analyses 5 capsules in singular (no duplicate counts) a difference of 30% can be detected instead of 64% with one capsule. When a laboratory analyses 10 capsules it can detect a difference of 44%. However, by analysing 10 capsules instead of 5 the detectable difference only decreases from 30% to 25%, almost no added benefit.

3.6.3.2. The number of replicates to be used

All figures and computations used above dealt with one replicate per capsule. In normal practice, when the capsule of high count CRM is dissolved in one litre of water or reconstituted in a peptone saline solution (*B. cereus*), several replicates can be performed. What is the influence of analysing more replicates per capsule on the power of the test? From a statistical point of view, Figure 3.1 shows that a benefit in power exists when passing from 1 to 2 replicates for one capsule. Figure 3.2 shows that for five capsules the added benefit is negligible. From both Figures it is clear that having 3 or 4 replicates is even of lower added value. Figure 3.3 shows the added value of having duplicate counts for 1 to 4 capsules. In this case the added value also decreases quickly. So, it does not make sense to analyse many replicates per capsule or many capsules in duplicate.

TABLE 3.5

LOWER AND UPPER GEOMETRIC MEAN VALUES AT A POWER OF AT LEAST 0.8 IN RELATION TO THE NUMBER OF CAPSULES AND REPLICATES EXAMINED USING THE *BACILLUS CEREUS* CRM 528 (MEYP AGAR AFTER 24 HOURS INCUBATION AT 30 °C, CERTIFIED VALUE 53.4).

Number of capsules examined	Number of replicates examined	Detectable difference in %	Lower geometric mean	Upper geometric mean
1	1	64	32	88
	2	45	36	78
2	1	43	37	77
	2	32	40	71
3	1	35	39	73
	2	27	42	68
4	1	31	40	70
	2	24	43	67
5	1	28	41	69
	2	22	43	66
6	1	26	42	68
	2	21	44	65
7	1	24	42	67
	2	20	44	64
8	1	23	43	66
	2	19	44	64
9	1	22	43	66
	2	18	45	64
10	1	21	44	65
	2	18	45	63

3.6.3.3. Practical results

In Table 3.5 an overview is presented of *the differences in percentage that can be detected* when analysing a certain number of capsules or replicates per capsule for *Bacillus cereus* (CRM 528) on MEYP agar using the ISO 7932 method with 24 h incubation time. The percentage of difference is computed from whatever value is the lowest, the certified value or the true laboratory geometric mean. For example, when three capsules with two replicates are analysed and the geometric mean obtained by the user is inbetween the lower and upper 95% confidence limits, there is no reason to assume that the true laboratory geometric mean deviates from the certified value. But, one has to bear in mind that only true laboratory geometric means of 42 or lower and 68 or higher (a minimum difference of 27%) can be detected (according to the power of 0.2 they are detectable on 80% of the occasions they occur). So, there are still rather high probabilities that the true laboratory geometric mean deviates from the certified value and that this will not be detected.

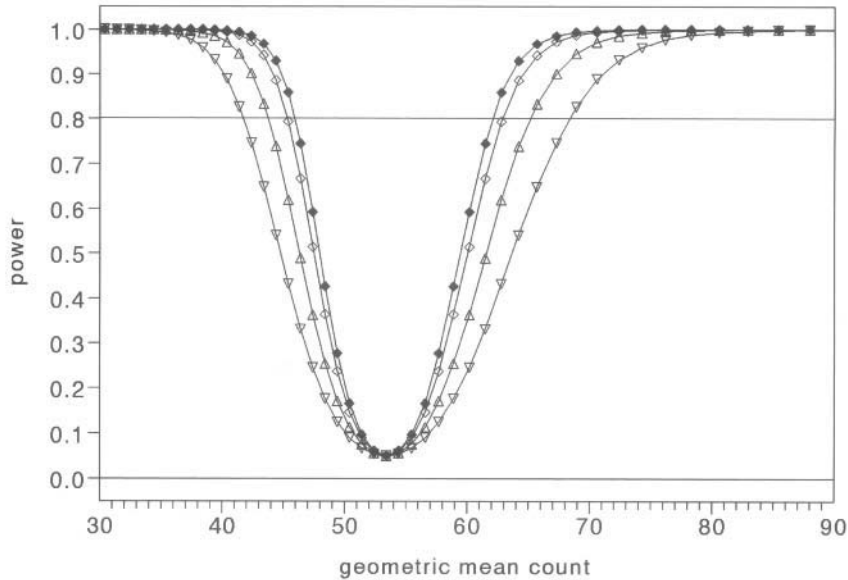


Fig. 3.2. Relationship between the power, the geometric mean and the number of replicates ($J = 1 \nabla$; $J = 2 \triangle$; $J = 3 \diamond$; $J = 4 \blacklozenge$) for the enumeration of 5 capsules of *Bacillus cereus* CRM (MEYP agar after 24 h incubation at 30°C, certified value 53.4).

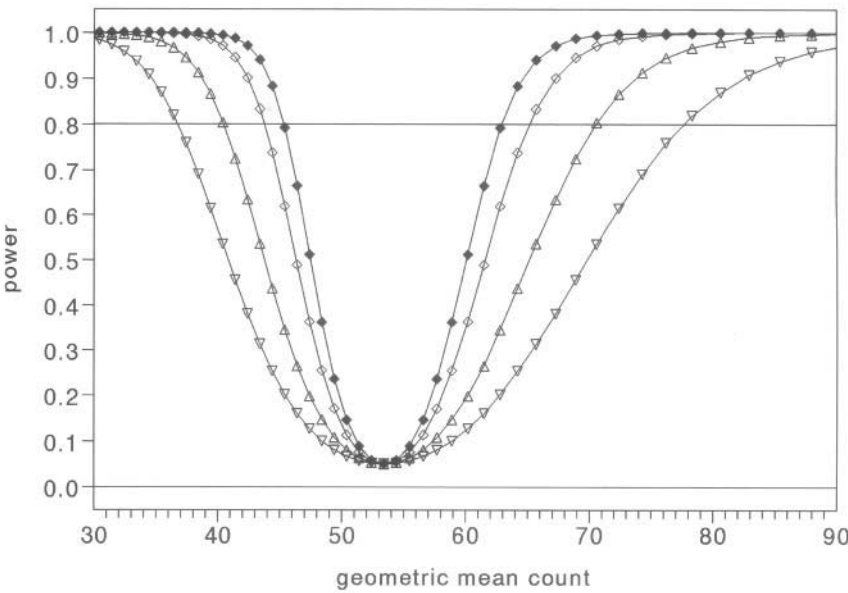


Fig. 3.3. Relationship between the power of the test, the geometric mean count and the number of capsules ($I = 1 \nabla$; $I = 2 \triangle$; $I = 3 \diamond$; $I = 4 \blacklozenge$) examined, using two replicates per capsule for the *Bacillus cereus* CRM 528 enumerated on MEYP agar after 24 h incubation at 30°C (certified value 53.4).

For every high level CRM, tables like Table 3.5 can be given. They give the differences that can be detected, for a specified number of capsules and replicates. From such tables, it is clear what the limitations are on detecting deviations from the certified value. With these limitations in mind a laboratory can decide on the number of capsules and replicates to use. For *Bacillus cereus* (CRM 528) on MEYP agar using the ISO 7932 method (24 h incubation), it is advisable to analyse 3 capsules and 2 replicates per capsule. In Table 3.6 an overview can be found of the recommended number of capsules and replicates to be analysed for all high counts BCR-CRMs. There is a lower limit for the difference that can be detected. How this limit can be computed is explained in the report published by J.A. van Dommelen [12]. For *Bacillus cereus* (CRM 528) on MEYP agar using the ISO 7932 method (24 h incubation) this implies that when hypothetically an infinite number of capsules and replicates is analysed, the lowest attainable difference is 13%. In Table 3.7 the lower limits for the differences that can be detected are shown for all high level BCR-CRMs. This lower limit depends mainly on the interlaboratory variance in the certification study.

3.6.4. Low level certified reference materials

3.6.4.1. Problem description

Low level certified reference materials are specially developed to see if a user is able to detect very small numbers of cfp of a micro-organism. In fact they allow one to validate a detection test. The micro-organisms concerned are pathogens that are not allowed to be present in food or water even at very low levels.

TABLE 3.6

RECOMMENDED NUMBER OF CAPSULES AND REPLICATES TO BE ANALYSED AND CORRESPONDING DETECTABLE DIFFERENCE (IN PERCENTAGES OF ALL COUNTS) FOR VARIOUS CRMS

species / CRM / method	number of capsules	number of replicates	difference
<i>Enterococcus faecium</i> (CRM506) on YA	3	2	46%
<i>Enterococcus faecium</i> (CRM506) on KFA	4	2	51%
<i>Enterococcus faecium</i> (CRM506) on m-EA	4	2	39%
<i>Enterobacter cloacae</i> (CRM527) on LSA	5	2	105%
<i>Bacillus cereus</i> (CRM528) on MEYP after 48 hours incubation	5	2	21%
<i>Bacillus cereus</i> (CRM528) on MEYP after 24 hours incubation	5	2	22%
<i>Bacillus cereus</i> (CRM528) on PEMBA after 48 hours incubation	5	2	26%
<i>Bacillus cereus</i> (CRM528) on PEMBA after 24 hours incubation	5	2	26%

TABLE 3.7

LOWEST DIFFERENCE (IN PERCENTAGE) THAT CAN BE DETECTED FOR ALL HIGH COUNT CRMS USING AN INFINITE NUMBER OF CAPSULES

species / CRM / method	lower limit
<i>Enterococcus faecium</i> (CRM506) on YA	41%
<i>Enterococcus faecium</i> (CRM506) on KFA	46%
<i>Enterococcus faecium</i> (CRM506) on m-EA	32%
<i>Enterobacter cloacae</i> (CRM527) on LSA	95%
<i>Bacillus cereus</i> (CRM528) on MEYP after 48 hours incubation	12%
<i>Bacillus cereus</i> (CRM528) on MEYP after 24 hours incubation	13%
<i>Bacillus cereus</i> (CRM528) on PEMBA after 48 hours incubation	17%
<i>Bacillus cereus</i> (CRM528) on PEMBA after 24 hours incubation	18%

When a material has been prepared to contain only a few cfp, it is normal that, due to the homogeneity limits of the procedure and the material composition, some capsules contain a few cfp and some others contain no cfp at all. The laboratory classifies the capsules containing cfp as positive. Those containing no cfp are classified as negative. The certificate of analysis of a CRM contains tables with the expected minimum number (with a 95% probability) of positive isolations for a certain number of capsules analysed. The optimal number of capsules that a laboratory should use is not mentioned in the certificate of the CRM. For low level (C)RMs replicates are not possible as the capsule is used as a whole. It is necessary to determine the number of capsules that allows a good evaluation of the performance of the method in the user's laboratory. This number of experiments must remain economically sustainable. This can be done with statistical methods as will be explained with examples below.

3.6.4.2. Theory of testing

In principle, the theory used for the presence-absence testing is the same as for counting large numbers of cfp, but differs in its application. Low count materials are characterised by the fact that some capsules contain no organisms. Because the certified value is in reality a fraction of negatives (capsules without bacteria), the fraction of negative isolations in the laboratory will be compared to the certified number of negatives.

A true fraction of negatives for a laboratory, π_{lab} , exists for the studied testing method. To determine this π_{lab} exactly, a very large number of capsules should be analysed. In practice only a small number of capsules can be analysed. With the results of this small number of capsules an estimate, called p_{lab} , of the fraction of negatives for the laboratory can be computed. This p_{lab} is an estimate of the unknown π_{lab} . The estimate, p_{lab} , is compared to the certified fraction π_{cert} . From this comparison, conclusions can be drawn about π_{lab} . In practice, the number of positive isolations

of the laboratory is compared to the minimum expected number of positive isolations (95% probability) defined by the certified fraction in the certificate. The laboratory concludes that π_{lab} is compatible with π_{cert} if its number of positive isolations is equal to or higher than the minimum expected number. It is possible that a laboratory finds more negatives — thus false negatives — than foreseen, i.e. $\pi_{lab} > \pi_{cert}$. Two situations are assumed to be possible: $\pi_{lab} = \pi_{cert}$ and $\pi_{lab} > \pi_{cert}$. Again, the conclusions drawn from the experiment do not always correspond with the real situation as shown in Table 3.8. The rest of the theory is the same as for high level CRMs. The probability for a type II error is the probability of concluding from the experiment that $\pi_{lab} = \pi_{cert}$, when in reality $\pi_{lab} > \pi_{cert}$. The power, $1 - \beta$, is the probability of detecting poor performance.

3.6.4.3. The number of capsules to use

The required number of capsules to be used depends on how small the difference is between the certified fraction of negatives and the true laboratory fraction of negatives that must be detected. It also depends on α and β . Expressed into health and safety considerations, it means: the smallest difference acceptable, which is of sufficient microbiological importance that it would be undesirable to fail to detect it. Large differences between the certified fraction and a true laboratory fraction of negatives can be detected with quite small numbers of capsules. To illustrate this the CRM with *Listeria monocytogenes* (CRM 595) will be considered. A certificate of CRM 595 is shown in Annex 3.4. The certified fraction of negatives for the presence-absence procedure based on the IDF method 143 is 1.2% [15]. When the laboratory analyses 10 capsules a true

TABLE 3.8

USE OF LOW CONTAMINATION LEVEL CERTIFIED REFERENCE MATERIALS IN MICROBIOLOGY. THEORY OF TESTING THE METHODS: TYPE II ERROR AND POWER OF THE TEST.

A IS THE PROBABILITY OF CONCLUDING INCORRECTLY THAT THE RESULT OF THE TEST *MLAB* DIFFERS SIGNIFICANTLY FROM THE CERTIFIED VALUE *MCERT*. B IS THE PROBABILITY THAT THE TYPE II ERROR OCCURS.

		Conclusion based on experiment	
		$\pi_{lab} = \pi_{cert}$	$\pi_{lab} > \pi_{cert}$
Reality	$\pi_{lab} = \pi_{cert}$	Correct! $1 - \alpha$	Type I error α
	$\pi_{lab} > \pi_{cert}$	Type II error β	Correct! $1 - \beta$
			↑ POWER

laboratory fraction of negatives of 28% can be detected (power of 0.8). This can be concluded from Figure 3.4. There is a large difference between the certified fraction of 1.2% and this true laboratory fraction of 28%. From Figure 3.4, it can also be concluded that a true laboratory fraction of negatives of 15% can be detected with 20 capsules and that with 30 capsules no gain is noticed.

3.6.4.4. Practical results

Once a laboratory has decided which fraction of negatives is acceptable for a given CRM and its certified fraction of negatives, it is possible to calculate the number of capsules that must be analysed. In Table 3.9 an overview is given of the required number of capsules to be analysed and to be found positive (for a power of 0.8 and $\alpha = 0.05$) to detect a certain laboratory fraction of negatives. For $\pi_{lab} = 3\%$ it should analyse at least 378 capsules with at least 370 positives. This can of course hardly be realised in practice. Table 3.9 shows that many capsules have to be analysed to detect small differences between π_{lab} and π_{cert} .

So, there are still rather high probabilities that the true laboratory fraction of negatives deviates considerably from the certified fraction and that this will not be detected.

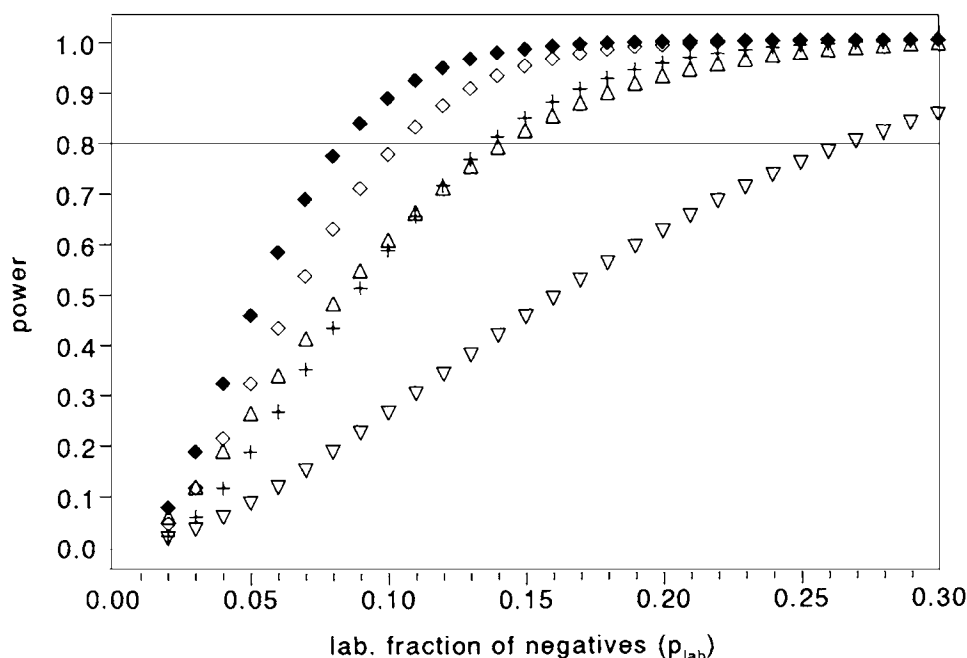


Fig. 3.4. Relationship between the power of the test, the number of capsules examined ($I = 10$ ∇ ; $I = 20$ \triangle ; $I = 30$ $+$; $I = 40$ \diamond ; $I = 50$ \blacklozenge) and the fraction of negatives found by the laboratory (p_{lab}) for a certified fraction of negatives (p_{neg}) of 0.012 for the *Listeria monocytogenes* CRM

TABLE 3.9

RECOMMENDED NUMBER OF CAPSULES AND MINIMUM NUMBER OF CAPSULES TO BE FOUND POSITIVE FOR *L. MONOCYTOGENES* TO BE ABLE TO DETECT A CERTAIN LABORATORY FRACTION OF NEGATIVES (π_{LAB}) WITH A POWER OF 0.80, FOR THE CERTIFIED FRACTION OF 1.2%.

π_{lab}	Recommended number of capsules to be examined	Minimum number of capsules to be found positive for <i>L. monocytogenes</i>
3%	378	370
4%	197	192
5%	110	107
6%	91	88
7%	60	58
8%	53	51
9%	47	45
10%	29	28
11%	27	26
12%	24	23
13%	23	22
14%	21	20
15%	19	18

A similar approach could be used for enumeration methods of low numbers of cfp. BCR recommends for incidental use of low level CRMs to analyse as many capsules as can be handled in one time, but a minimum of 20.

3.6.5. Frequency of use

CRMs for microbiology should follow the same type of use as in chemistry. The frequency depends also on similar parameters as in chemistry. There should be a strong emphasis on the fact that CRMs should certainly be used when new batches (from the same or another supplier) of culture media are used in the laboratory. Stability of production of the media is too uncertain to trust suppliers [16]. As the medium may strongly affect the accuracy of results and if the laboratory does not maintain its own strains to control its media it is advised to buy a CRM.

3.6.6. Conclusions

The above discussions on the use of CRMs in microbiology could be extended to all types of materials certified for a finite number of cfp and certified through a specific method. Further details on the statistical approach briefly described in the above sections can be found in the literature [12,16].

3.7. REFERENCES

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Annex 3.1

Comparison of precisions of measurement methods

In the certification exercise of trace elements in lichens (BCR-CRM 482) two or more laboratories applied the same technique of final determination for the same element. In such a case it was possible to compare the results per technique. A grand mean of the means of all the three or more laboratories applying the same technique of final determination was calculated. The obtained grand means were then compared to investigate whether a particular bias could be attributed to any method. The bar-graphs present the grand mean per technique of final measurement (for those elements where this was possible) together with the standard deviation of the set. The table presents the results of the evaluation.

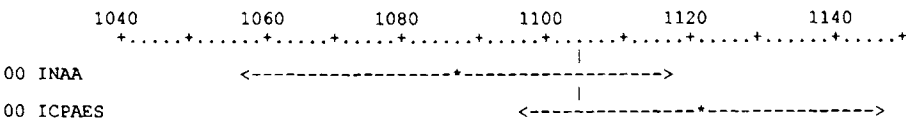
RESULTS OF THE EVALUATION ON CONSISTENCY OF THE METHODS

Element	Technique of final determination	CV (%) between means of laboratories with the same technique	number of sets of results	CV (%) between means of different techniques
Aluminium	INAA	2.8	2	2.3
	ICPAES	2.2	4	
Arsenic	INAA	5.4	2	9.2
	RNAA	7.1	2	
Chromium	INAA	2.2	2	2.2
	RNAA	4.7	3	
Copper	ICPAES	4.2	3	0.7
	ICPMS	2.4	2	
Mercury	ICPMS	3.4	3	1.2
	CVAAS	0.3	2	
Zinc	INAA	2.8	2	3.8
	ICPMS	1.8	3	
	ICPAES	3.3	4	

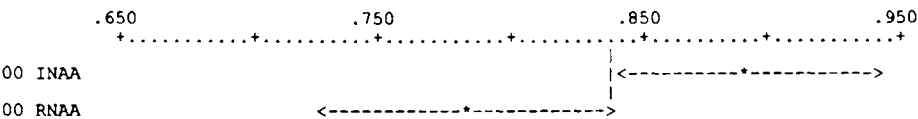
As shown in the table, the CVs within one method are generally of the same order of magnitude than those between different techniques. For ICPMS and ICPAES (copper and mercury), the larger CVs were due to the fact that the laboratories were operating close to the limits of determination of the methods.

Annex 3.1 (continued)

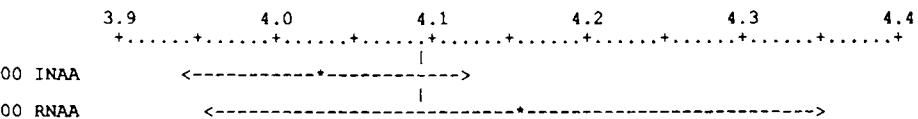
Aluminium



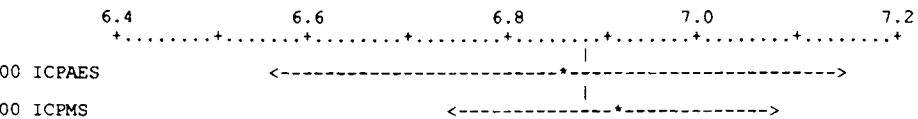
Arsenic



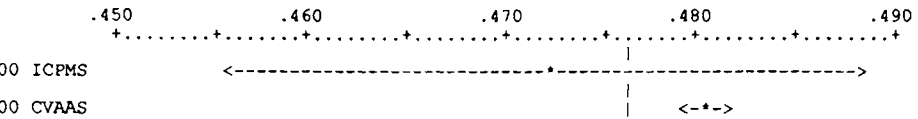
Chromium



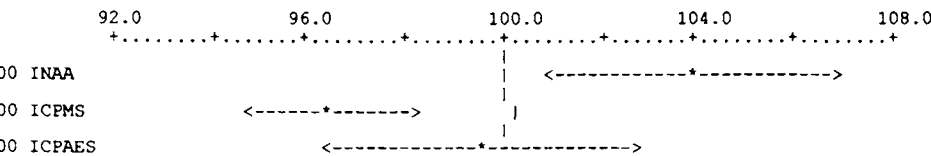
Copper



Mercury



Zinc



Annex 3.2

Various ways of using a BCR-CRM

Background

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans comprise groups of, respectively, 75 and 135 individual chemical compounds. These compounds are highly lipophilic and resistant to chemical breakdown. Therefore they tend to accumulate in the food chain. The relative toxicity for a number of PCDDs and PCDFs has been agreed internationally and the toxicity equivalency factors (I-TEF) are given in the table below. Multiplication of the concentration of a given compound by the corresponding I-TEF and summation of the values for all congeners allows the total PCDD and PCDF content to be expressed in a single International-toxicity equivalent (I-TEQ) value. Some countries have adopted a maximum residue limit (MRL) based on an I-TEQ total for cow's milk e.g. MRL 6 pg TEQ/g fat.

The milk powder of BCR-CRM 607 contains approximately 30% fat (mass fraction, on a dry mass basis). As can be seen from the table, 2,3,7,8 TCDD (D48) has the highest toxicity equivalent factor, thus having a major contribution to the total toxicity of the PCDD and PCDF in the milk. Therefore, the determination of D48 has to be highly accurate and in particular trueness is essential. The various ways of using the BCR-CRM 607 for checking trueness is described here.

THE SEVENTEEN 2,3,7,8,-SUBSTITUTED 'PCDDs AND PCDFs' AND THEIR I-TEF VALUES

PCDD or PCDF isomers		I-TEF
D48	2,3,7,8-TCDD	1
D54	1,2,3,7,8-PeCDD	0.5
D66	1,2,3,4,7,8-HxCDD	0.1
D67	1,2,3,6,7,8-HxCDD	0.1
D70	1,2,3,7,8,9-HxCDD	0.1
D73	1,2,3,4,6,7,8-HpCDD	0.01
D75	OCDD	0.001
F83	2,3,7,8-TCDF	0.1
F94	1,2,3,7,8-PeCDF	0.05
F114	2,3,4,7,8-PeCDF	0.5
F118	1,2,3,4,7,8-HxCDF	0.1
F121	1,2,3,6,7,8-HxCDF	0.1
F124	1,2,3,7,8,9-HxCDF	0.1
F130	2,3,4,6,7,8-HxCDF	0.1
F131	1,2,3,4,6,7,8-HpCDF	0.01
F134	1,2,3,4,7,8,9-HpCDF	0.01
F135	OCDF	0.001

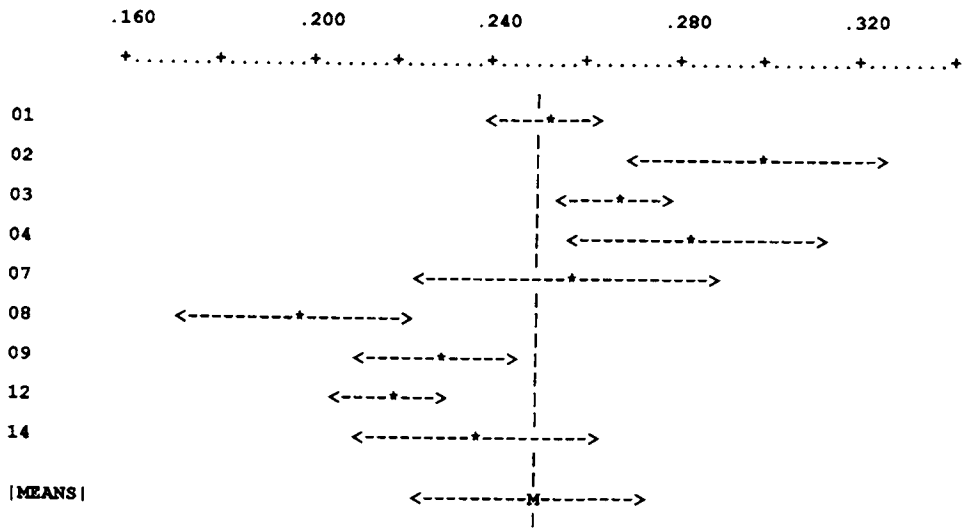
The table and the bar-graph presentation show all data retained for the certification of D48.

Annex 3.2 (continued)

INDIVIDUAL RESULTS USED FOR CERTIFICATION OF 2,3,7,8 — TCDD (D48) IN MILK POWDER BCR-CRM 607 EXPRESSED IN NG PER KG MILK POWDER

Lab	REPLICATES					MEAN	S.D.
01	.242	.258	.240	.245	.259	.249	.009
02	.290	.270	.290	.300	.330	.296	.022
03	.258	.264	.255	.266	.279	.264	.009
04	.270	.300	.270	.260	.310	.282	.022
07	.230	.240	.270	.290	.240	.254	.025
08	.201	.217	.208	.186	.167	.196	.020
09	.250	.220	.220	.220	.220	.226	.013
12	.210	.210	.230	.210	.220	.216	.009
14	.260	.210	.250	.220	.230	.234	.021

BAR-GRAPH FOR LABORATORY MEANS AND 95% CI



ISO Guide 33 — approach for trueness

Laboratory A

Let us assume that laboratory A which intends to test its performance has produced the following data with CRM 607:

0.20	0.18	0.17	0.16	0.22	0.18	0.17	0.21	0.22	0.20
mean \bar{X} :		0.19	s_m :	0.02	$\mu\text{g/kg}$				

certified value μ : 0.25 +/- 0.03 $\mu\text{g/kg}$

Annex 3.2 (continued)

The laboratory uses X and s_m and compares them to μ the certified value of the CRM. As can be seen the uncertainty of the certified value is 7 times lower than the reproducibility of the measurement method s_m . The comparison is done as follows:

$$-a_2 - 2s_m \leq X - \mu \leq a_1 + 2s_m$$

In the case of PCDD and PCDF measurements there is a maximum limit for the TEQ allowed for milk powder. This limit is of 6 pg TEQ/ g fat. The TEQ of CRM 607 is close to 6.3 pg TEQ/ g fat (1.9 pg TEQ/ g milk powder and a fat content of approx. 30%). Therefore, it would be logic to allow no adjustment factor for a_1 ($a_1 = 0$) as the method must be of the best accuracy versus higher values. For a_2 an adjustment factor can be allowed because no lower limit exists and because the determination of D48 becomes rapidly very difficult when the content decreases in a sample. Therefore, a_2 could be set equal to 2 or more.

Following ISO Guide 33, laboratory A would achieve the present performance:

$$-2 - (2 \times 0.02) \leq 0.19 - 0.25 \leq + (2 \times 0.02) \Leftrightarrow -2.04 \leq -0.06 \leq + 0.04$$

which is acceptable.

Laboratory B

Laboratory B has achieved the following results:

0.32	0.28	0.27	0.22	0.32	0.38	0.37	0.21	0.22	0.40
mean X :		0.30	s_m :	0.07	$\mu\text{g/kg}$				

the result of the test would be: $-2.14 \leq +0.05 \leq +0.14$, which is not acceptable following ISO Guide 33 and the above criteria.

In fact laboratory A has data close to those of laboratory 08 and laboratory B results close to those of laboratory 02 which participated both in the certification study. The results of both laboratories 02 and 08 were accepted for certification. Therefore, in the situation described above for laboratory B, our test following ISO Guide 33 with no adjustment factor for a_1 is 'more severe' than the certifying body.

Conclusion about the use of ISO Guide 33

Two conclusions could be drawn:

– a_1 should be set at 0.5 or more – the certified uncertainty is too small i.e. the 95% confidence interval is too optimistic.

The later case is a decision of the certifying body and must be taken as it is (C.I. is already more than 10%).

Alternative approach

The user could also use another way of checking his method. He could decide not to follow ISO Guide 33 and verify if its own values are covered by the population of data delivered in the certification exercise e.g. within the range of lab 02 and 08 taking their own standard deviation into account:

$$0.20 - 0.02 \leq X \leq 0.29 + 0.02 \Leftrightarrow 0.18 \leq X \leq 0.31$$

Annex 3.2 (continued)

Very simple but not unfair. As can be seen, in such a case the results of both Laboratories A and B would be acceptable. Such a use of BCR-CRM 607 is only possible if all individual data used for the certification are published in a report as it is the case for BCR materials.

Approach for precision

A similar game can be played with ISO Guide 33 for the verification of precision. CRM, in particular matrix materials for the validation of methods for environmental monitoring purposes, are more used for the assessment of trueness. The exercise on precision can also be performed with non certified RM. The CRM gives through the acceptance of widely differing methods the precision potential of these methods. This is discussed somewhat in section 3 (see Table 3.2).

Annex 3.3

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COMMUNITY BUREAU OF REFERENCE — BCR

CERTIFIED REFERENCE MATERIAL

CERTIFICATE OF ANALYSIS

CRM 528, number concentration of colony forming particles of <i>Bacillus cereus</i> in artificially contaminated milk powder				
Procedure	Certified value ^a $C_{B.cfp}/ml^{-1}$	95% confidence limits $C_{B.cfp}/ml^{-1}$		Sets of accepted results
		lower limit	upper limit	
MEYP (ISO 7932) after 24 h incubation	53.4	51.7	55.2	11 ^b
MEYP (ISO 7932) after 48 h incubation	53.7	52.1	55.4	11 ^b
PEMBA (L 00.00 – 25) ^d after 24 h incubation	55.0	52.8	57.4	11 ^c
PEMBA (L 00.00 – 25) ^d after 48 h incubation	55.8	53.6	58.0	11 ^c

a: this value is the geometric mean of n accepted sets of data, independently obtained by 11 laboratories

b: comprising the results of 154 capsules

c: comprising the results of 153 capsules

d: German Federal Food Law method number

DESCRIPTION OF THE MATERIAL

The CRM consists of 0.317 g artificially contaminated spray dried milk contained in an ochre/white gelatin capsule. The strain used for the contamination is *Bacillus cereus* (ATCC 9139). The capsules are packed in a plastic container with a silica gel dessicant bag.

STABILITY

The material showed no significant decrease or increase of the mean $C_{B.cfp}$ after 28 days of storage at 22°C and 30°C (enumeration on MEYP and SBA). However it is kept in stock

Annex 3.3 (continued)

at -20°C ; the stability of the stored material will be periodically monitored. Laboratories will be informed in case significant deviations from the certified value is observed.

INSTRUCTIONS FOR USE

On receipt store the capsules at $(-20 \pm 5)^{\circ}\text{C}$. If only a few are used return the closed container with the bag of desiccant to $(-20 \pm 5)^{\circ}\text{C}$ immediately. The remaining capsules may be used up to the expiry date.

DO NOT TOUCH THE CAPSULE BY HAND, USE STERILE FORCEPS OR WEAR STERILE GLOVES. THE CAPSULES SHOULD NOT BE OPENED.

Reconstitute one capsule in 10 ml peptone saline solution according to Annex E of the certification report.

Use only completely reconstituted capsules. No lumps should be visible with the naked eye.

After reconstitution, place the tube(s) with the solution directly in melting ice and use the material within 3 hours, leaving it in melting ice. If the tubes are not cooled sufficiently growth can occur within a short period of time (see also Chapter 9 of the certification report).

INFORMATION FOR USE

The materials can be used for:

- a. Checking the spread plate technique according to ISO 7932 (1987) using Mannitol Egg Yolk Polymyxin agar (MEYP).

For this purpose pipette (0.10 ± 0.002) ml of the cooled capsule solution onto the surface of a MEYP plate and spread the inoculum as quickly as possible over the surface by means of a sterile spreader trying not to touch the sides of the plate. Incubate at $(30 \pm 1)^{\circ}\text{C}$ for (48 ± 4) hours. Count the number of colonies after (24 ± 2) h and/or after (48 ± 4) h.

- b. Checking the spread plate technique according to the German Federal Food Law method no L 00.00 – 25 (1992) using Pyruvate Egg yolk Mannitol Bromothymol blue Agar (PEMBA).

For this purpose pipette (0.10 ± 0.002) ml of the cooled capsule solution onto the surface of a PEMBA plate and spread the inoculum as quickly as possible over the surface by means of a sterile spreader trying not to touch the sides of the plate. Incubate at $(37 \pm 1)^{\circ}\text{C}$ for (48 ± 4) hours. Count the number of colonies after (24 ± 2) h and/or after (48 ± 4) h.

- c. Checking the spread plate technique according to the Nordic Committee on Food Analysis method no 67, second edition (1982) using Sheep Blood Agar (SBA).

Annex 3.3 (continued)

For this purpose pipette (0.10 ± 0.002) ml of the cooled capsule solution on the surface of a SBA plate and spread the inoculum as quickly as possible over the surface by means of a sterile spreader trying not to touch the sides of the plate.

Incubate at (30 ± 1)°C or at (37 ± 1)°C for (24 ± 2) hours.

In Tables 1 and 2 the confidence limits (geometric mean $C_{B,cfp}/\text{ml}^{-1}$) for the most frequently used combinations of capsules and replicates are given for MEYP and PEMBA respectively. For the spread plate technique on SBA only indicative values based on 4 sets of data are given in Chapter 8 of the certification report.

More information on the use of this CRM can be found in Chapter 9 of the certification report.

PARTICIPATING LABORATORIES**Preparation**

- | | | |
|---|-----------|----|
| — Laboratory of Water- and Food Microbiology, National
Institute of Public Health and Environmental Protection | Bilthoven | NL |
|---|-----------|----|

Identification

- | | | |
|--|-----------|----|
| — Food Hygiene Laboratory, Food and Enteric Reference
Division, Central Public Health Laboratory | London | GB |
| — Laboratory for Bacteriology and Antimicrobial Agents,
National Institute of Public Health and Environmental
Protection | Bilthoven | NL |
| — Laboratory of Water- and Food Microbiology, National
Institute of Public Health and Environmental Protection | Bilthoven | NL |

Homogeneity

- | | | |
|---|-----------|----|
| — Laboratory of Water- and Food Microbiology, National
Institute of Public Health and Environmental Protection | Bilthoven | NL |
|---|-----------|----|

Stability

- | | | |
|---|-----------|----|
| — Laboratory of Water- and Food Microbiology, National
Institute of Public Health and Environmental Protection | Bilthoven | NL |
|---|-----------|----|

Analyses

- | | | |
|---|---------------------|----|
| — Microbiology Department, Campden Food & Drink
Research Association | Chipping
Campden | GB |
|---|---------------------|----|

Annex 3.3 (continued)

— Department of Microbiology, Central Institute for Nutritional Research	Zeist	NL
— Institut für Lebensmittelhygiene, Free University of Berlin	Berlin	DE
— Istituto di Tecnologie Alimentari	Udine	IT
— Laboratório Nacional de Engenharia e Tecnologia Industrial	Lisbon	PT
— Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food	Weybridge	GB
— Laboratory of Water- and Food Microbiology, National Institute of Public Health and Environmental Protection	Bilthoven	NL
— State Institute for the Inspection of Agricultural Products	Wageningen	NL
— Rijkszuivelstation	Melle	BE
— Istituto di malattie infettive, Profilassi E Polizia Veterinaria, Università di Bologna	Bologna	IT
— Department of Food Microbiology, University of Bonn	Bonn	DE
— Laboratoire Central d'Hygiène d'Alimentaire, Ministère de l'Agriculture	Paris	FR

METHODS USED IN CERTIFICATION STUDY

- Spread plate technique according to ISO 7932 (1987) using the Mannitol Egg Yolk Polymyxin agar (MEYP).
- Spread plate technique according to the German Federal Food Law method no L 00.00 – 25 (1992) using Pyruvate Egg yolk Mannitol Bromothymol blue Agar (PEMBA).
- Spread plate technique according to the Nordic Committee on Food Analysis method no 67, second edition (1982) using Sheep Blood Agar (SBA).

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NOTE

A technical report giving details of the methods and results is supplied with each first delivery of the CRM.

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Annex 3.3 (continued)

TABLE 1.

THE 95% CONFIDENCE LIMITS OF THE CERTIFIED VALUE ON MEYP AFTER 24 H AND 48 H INCUBATION AS GEOMETRIC MEAN $C_{B,CFP}/ML^{-1}$

number of capsules analysed	24 h incubation				48 h incubation			
	lower limit		upper limit		lower limit		upper limit	
	1 ^a	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a
1	38	41	76	70	38	41	76	70
2	41	44	69	65	42	44	69	65
3	43	45	66	63	44	46	66	63
4	44	46	65	62	45	46	65	62
5	45	46	64	61	45	47	64	62
6	45	47	63	61	46	47	63	61
7	46	47	62	61	46	47	62	61
8	46	47	62	60	47	48	62	61
9	46	47	62	60	47	48	62	60
10	47	48	61	60	47	48	61	60
11	47	48	61	60	47	48	61	60
12	47	48	61	60	47	48	61	60

a: number of replicates

TABLE 2.

THE 95% CONFIDENCE LIMITS OF THE CERTIFIED VALUE ON PEMBA AFTER 24 H AND 48 H INCUBATION AS GEOMETRIC MEAN $C_{B,CFP}/ML^{-1}$

number of capsules analysed	24 h incubation				48 h incubation			
	lower limit		upper limit		lower limit		upper limit	
	1 ^a	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a
1	39	41	78	74	39	42	80	75
2	42	44	71	69	43	45	73	70
3	44	45	69	67	45	46	70	67
4	45	46	67	46	47	68	66	
5	46	47	66	65	46	47	67	66
6	46	47	66	64	47	48	67	65
7	46	47	65	64	47	48	66	65
8	47	47	65	64	47	48	66	64
9	47	48	65	64	48	48	65	64
10	47	48	64	63	48	49	65	64
11	47	48	64	63	48	49	65	64
12	47	48	64	63	48	49	65	64

a: number of replicates

Annex 3.4

COMMISSION OF THE EUROPEAN COMMUNITIES

COMMUNITY BUREAU OF REFERENCE — BCR

CERTIFIED REFERENCE MATERIAL

CERTIFICATE OF ANALYSIS

CRM 595, number fraction of negative capsules and number of cfp of <i>Listeria monocytogenes</i> in a capsule containing artificially contaminated milk powder				
quantity (procedure)	certified value	95% confidence limits of z		sets of accepted results
		lower limit	upper limit	
mean number of <i>L. monocytogenes</i> in one capsule (z) (enumeration procedure)	7.2 ^a	6.8 ^b	7.6 ^b	11 ^d
fraction of capsules in which no <i>L. monocytogenes</i> could be detected (enumeration procedure)	0.075%	0.050% ^b	0.11% ^b	11 ^d
fraction of capsules in which no <i>L. monocytogenes</i> could be detected (presence/absence procedure according to IDF standard 143)	1.2%	0	2.3% ^c	12 ^e

- a: this value is the arithmetic mean of 11 accepted sets of data independently obtained by 11 laboratories
- b: two sided 95% confidence interval
- c: one sided 95% confidence upper limit
- d: comprising the results of 549 capsules, obtained by 11 laboratories
- e: comprising the results of 564 capsules, obtained by 12 laboratories

DESCRIPTION OF THE MATERIAL

The CRM consists of 0.34 g artificially contaminated spray dried milk contained in an orange/white gelatin capsule. The strain used for the contamination is *Listeria monocytogenes* (Scott A strain). The capsules are packed in a plastic container with a silica gel dessicant bag.

STABILITY

The material showed a significant decrease of the mean z after 38 days of storage at 5°C and 22°C (enumeration procedure). Also at -20°C of the long term stability test a

Annex 3.4 (continued)

small decrease of the mean of z was observed. The stability of the stored material will be periodically monitored. Laboratories will be informed in case significant deviations from the certified value is observed. Due to the limited stability of the material it is necessary to ship the materials by special delivery service to eliminate any possible effects of transport conditions on z .

Brussels, November 1995

BCR for certified true copy

INSTRUCTIONS FOR USE

The materials were shipped in a polystyrene box and were kept cool by means of cooling devices. A cold-chain monitor was included in the parcel for recording of the temperature during transport. Check this monitor directly after receipt of the parcel. Compartments B, C and D must not show any discolouration (blue colour), if so, please contact the shipper directly. Compartment A may be coloured blue. Coloration of this compartment means that the temperature during shipment was greater than 10°C but less than 34°C for a short period of time. This should, however, have no effect on the results obtained with the materials. More information on the cold chain monitor is presented in annex C of the report.

One container holds 10 capsules. If only a few capsules are used, the remaining capsules can still be used, if stored in the closed container, with the bag of desiccant, and returned to $(-20 \pm 5)^\circ\text{C}$ immediately.

Do not touch the capsules by hand, USE sterile forceps or wear sterile gloves. The capsules should not be opened.

INFORMATION FOR USE

The material is intended for the evaluation of the performance of IDF procedures for the detection of *L. monocytogenes*.

Each capsule is aseptically added as a whole to a jar containing the enrichment broth. The broth must be between 20°C and 38°C before a capsule is added. The jars are handled further according to the IDF procedure.

Table 1 presents the recommended number of capsules to be examined and the minimum number of capsules to be found positive for *L. monocytogenes*, in relation to the fraction of negatives a laboratory works with (called π_{lab}) for the certified fraction of 1.2%. A laboratory has to choose which π_{lab} it wants to be able to detect. The more capsules that can be examined the better the π_{lab} can be tested against the certified fraction of negatives. A laboratory has to find an optimum between the π_{lab} it wants to be able to detect and the number of capsules that it can examine at the same time.

More details on the recommendations for the number of capsules to be examined is presented in Annex M of the report. The method for the calculation of the minimum number of capsules to be found positive for *L. monocytogenes* is presented in Annex N of the report.

Annex 3.4 (continued)

TABLE 1

RECOMMENDED NUMBER OF CAPSULES AND MINIMUM NUMBER OF CAPSULES TO BE FOUND POSITIVE FOR *L. MONOCYTOGENES* TO BE ABLE TO DETECT A CERTAIN LABORATORY FRACTION OF NEGATIVES (π_{LAB}) WITH A POWER OF 0.80, FOR THE CERTIFIED FRACTION OF 1.2%.

π_{lab}	Recommended number of capsules to be examined	Minimum number of capsules to be found positive for <i>L. monocytogenes</i>
3%	378	370
4%	197	192
5%	110	107
6%	91	88
7%	60	58
8%	53	51
9%	47	45
10%	29	28
11%	27	26
12%	24	23
13%	23	22
14%	21	20
15%	19	18

For example: A laboratory wants to be able to detect a π_{lab} of 10%. Using Table 13 the laboratory needs to examine 29 capsules at one time. Finding 27 positives or less out of the 29 capsules examined indicates that the laboratory finds more negatives than can be expected (based on the certified value and the number of capsules examined). Finding 28 or 29 positives indicates that there is no reason to assume that the laboratory works with a π_{lab} that deviates from the certified fraction of negatives (remember that only a deviation from π_{lab} of 10% or more could be detected in this case).

The material can also be used for testing the enumeration of *L. monocytogenes*. For the determination of *z* the capsules have to be enumerated according to a strict protocol as described in annex E of the report (Enumeration of *L. monocytogenes* in reference materials for food microbiology). It is essential that the capsules are completely dissolved as otherwise the *z* is underestimated. The number of capsules to be enumerated should be ca. 50. The expected contamination level to be found will then be 7.2 ± 1.3 .

PARTICIPATING LABORATORIES

Preparation

- Laboratory of Water- and Food Microbiology, National Institute of Public Health and Environmental Protection Bilthoven NL

*Annex 3.4 (continued)***Identification**

- | | | |
|--|-----------|----|
| — Food Hygiene Laboratory, Central Public Health Laboratory | London | GB |
| — Laboratory for Bacteriology and Antimicrobial Agents,
National Institute of Public Health and Environmental
Protection | Bilthoven | NL |

Identification

- | | | |
|---|-----------|----|
| — Laboratory of Water- and Food Microbiology, National
Institute of Public Health and Environmental Protection | Bilthoven | NL |
|---|-----------|----|

Identification

- | | | |
|---|-----------|----|
| — Laboratory of Water- and Food Microbiology, National
Institute of Public Health and Environmental Protection | Bilthoven | NL |
|---|-----------|----|

Identification

- | | | |
|---|---------------------|----|
| — Microbiology Department, Campden & Chorleywood
Food Research Association | Chipping
Campden | GB |
| — Department of Microbiology, Central Institute for
Nutritional Research | Zeist | NL |
| — Institut für Lebensmittelhygiene, Free University of Berlin | Berlin | DE |
| — Istituto di Tecnologie Alimentari | Udine | IT |
| — Laboratório Nacional de Engenharia e Tecnologia Industrial | Lisbon | PT |
| — Central Veterinary Laboratory, Ministry of Agriculture,
Fisheries and Food | Weybridge | GB |
| — Laboratory of Water- and Food Microbiology, National
Institute of Public Health and Environmental Protection | Bilthoven | NL |
| — State Institute for the Inspection of Agricultural Products | Wageningen | NL |
| — Rijkszuivelstation | Melle | BE |
| — Istituto di malattie infettive, Profilassi E Polizia Veterinaria,
Università di Bologna | Bologna | IT |
| — Department of Food Microbiology, University of Bonn | Bonn | DE |
| — Laboratoire Central d'Hygiène d'Alimentaire, Ministère
de l'Agriculture | Paris | FR |

METHODS USED IN CERTIFICATION STUDY

- a. To determine the presence or absence of *L. monocytogenes* in a single capsule, the IDF standard 143 for the detection of *L. monocytogenes* was used (see annex G of the report).
- b. For the enumeration of *L. monocytogenes* a specifically developed procedure, as described in annex E of the report, was used.

*Annex 3.6 (continued)***LEGAL NOTICE**

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NOTE

A technical report giving details of the methods and results is supplied with each first delivery of the CRM.

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*Chapter 4***Production of matrix CRMs****4.1. GENERAL CONSIDERATIONS AND WARNINGS**

Many aspects of the production of CRMs are valid for simple RMs, e.g. laboratory RMs, samples used in interlaboratory studies, etc. The main differences are found in the actual certification of the assigned values, the extent of additional guarantees that usually accompany the certified value (stability and homogeneity data etc.), often the size of the produced batch, and finally the existence of a certifying body and the supply service.

Therefore, analysts willing to prepare RMs for intralaboratory purposes (e.g. to set up control charts) will find useful information and advice in this chapter. The degree of attention and effort, i.e. the amount of time and consequent economic resources to be devoted to the preparation of an own RM will decide the quality of the RM and its usefulness in the laboratory's quality system. Quality always pays. Therefore, we would suggest that the laboratory management encourages those in charge of preparing or purchasing RMs to allow a maximum of quality for these strategic materials. The general quality of the analytical work performed and, consequently, the status of the laboratory, strongly depend on them. Equally, we would recommend auditors in certification and accreditation not only to recommend the use of RMs and CRMs but also to devote time and attention to the way the laboratory has prepared the RMs and how they use them. This is equally important as the maintenance of primary calibration materials.

Unfortunately, quality criteria and in particular mandatory quality requirements for the production and producers of RMs and CRMs are not yet available. Only ISO Guides (ISO Guide 34) exist, which are not mandatory. This may change in future.

4.2. PREPARATION OF THE MATERIAL**4.2.1. Choice of the material**

The (C)RM has to fulfil a defined task. Therefore, the material must be accurately chosen. The selection of the material itself is easy when pure substances for calibration or identification purposes are considered. For artificial materials such as manufactured products e.g. steel, alloys, plastics, ceramics etc., the manufacturing process may be the defining tool. Where natural matrix materials are concerned, the selection of the (C)RM passes through a careful study of the objective of the method to be validated. A method for contaminated soil analysis has to cover soils of various origin, the CRM(s) to validate

the accuracy or the RM(s) for statistical control have to take into account the influence of the variability of the samples and the effect on the method itself. The (C)RM must be at least as difficult to analyse as the most difficult samples. The representativeness of the material is the first quality parameter a (C)RM must demonstrate. Any action on the material should respect this representativeness. Because of this fundamental requirement it is obvious that manufacturing of (C)RMs cannot be the task of a 'specialised' laboratory only. Production of (C)RMs must involve the end-users of the material, as well as metrologists and material science specialists for the technical work. The representativeness for a reference material used for chemical analysis is based on:

- the substances to be measured and their respective concentration in the material;
- the matrix of the material;
- how the analytes are bound to the matrix;
- the presence of potential interfering substances;
- the physical status of the material.

4.2.1.1. Analytes and their concentration

It is logical that the substances to be monitored have to be present in the material. These substances must also be present in the appropriate chemical form (type of salt, valence, etc.) and in concentrations close to what is measured on daily routine samples. The latter requirement is logical when dealing with speciation measurements (organics, organo-metallics etc.). It is also important for trace element determinations as the chemical form of the elements may have a strong influence on the analytical procedure, i.e. the sample pretreatment. A classical example can be found in the determination of traces of As in marine animal tissues. Arsenobetaine represents often more than 50% of the total As present in fishes. This compound is difficult to destroy chemically, even with hot aqua regia. Therefore, when validating spectrometric methods for the total As content, the (C)RM must contain arsenobetaine to be representative [1–4]. This example also stresses the precautions that must be taken when producing artificial — spiked — samples (standard addition method) or reference materials. The concentration pattern of the various substances is also of primary importance. When several compounds or elements have to be monitored in daily work, and the samples represent wide ranges of concentrations, this must be reflected by the reference material: the (C)RM should reflect the most critical situation.

The presence of interfering substances affects also the level of representativeness of the (C)RM. The identity and content of these substances both play a role. Present as traces, they do not affect the result of the determination of a compound. Present at high concentrations, they may drastically affect the detection, the resolution of a chromatographic separation, the integration of peaks etc. In some circumstances and in particular when (C)RMs are used to validate methods foreseen for regulatory purposes, materials must be enriched to achieve the required levels of contents of analyte and interfering substances. For the quality control of methods for the organo-chlorine pesticides (OCP) content in animal fat, the BCR has produced a CRM [5,6] with incurred pesticides. Pigs were fed with feed enriched with a series of OCP. The pesticide levels in the daily feed were calculated for a diet of one year after having established

the concentration behaviour of the animal's metabolism in the fat tissue. The levels certified are very close to the limits set in the European Directive [7], which demonstrates the accuracy of preparation [8]. Other similar approaches have been used; the limit of the approach can be set by safety, environmental, or ethical concerns.

4.2.1.2. The matrix of the material

All analysts know that the difficulty of a chemical measurement lies as much in the substance to be measured as in the matrix in which it is hidden. The influence of the matrix will be eliminated by the pretreatment steps of the method. Therefore, a good (C)RM has a matrix similar to the daily routine samples. The reference material must pose at least as much and similar analytical difficulties as the routine samples. Two soils, e.g. a sandy soil and clay soil, behave very differently in a digestion or extraction step. This explains why BCR produced various soil reference materials [9–11]. The same remarks are valid for sediments, plant materials (silicate content, presence of waxes etc.) or animal tissues (various contents of fatty tissues).

The way the analytes are bound to the matrix components is directly linked to the matrix status. This status will be changed by the treatment the matrix will undergo during the preparation of the material, e.g. drying. The physical binding must be addressed when producing artificially-enriched materials. Chlorinated pesticides (DDT, drins etc.) in organic matter of a soil or sediment that was contaminated a long time ago are different to the same substances after having been spiked on the matrix. The most difficult situations are encountered with incurred substances, e.g. those that have been subject to animal or plant metabolism. It is impossible to try to copy artificially such situations. It is also not realistic, at least economically, to determine all the components of a matrix and produce a fully artificial material. It is best to collect natural samples and to seek advice from a professional in the field.

4.2.1.3. The physical status

The physical status of the material has also a strong influence on the representativeness but it is often one of the concessions to be made by the user and the producer to obtain stable and homogeneous samples. Therefore, the (C)RM producer will often dry the material, sterilise or stabilise it. Drying and sterilisation can affect strongly the material's status and behaviour in the analytical process. It can also change its composition. When clay-rich soil is dried, the clay layers stick together and consequently traces of substances may be trapped and are no longer accessible to an extracting solvent. The material must be pre-wetted before use. Other examples are famous for the change in behaviour after drying, e.g. fresh and powdered milk, mussel tissues. Treatments other than drying can affect the representativeness of materials, e.g. fresh/de-frozen serum. Fresh food materials containing microbes for the quality control of microbiological methods cannot be produced within the actual knowledge. Such fresh materials are not stable for prolonged periods of time. The matrix will suffer degradation and the microbes will multiply or die. In fresh materials, prepared for chemical monitoring, the microbial activity may alter the substance to be measured or the matrix itself. It must be systematically studied

how much a material is affected in its behaviour by undergoing a treatment procedure. Sometimes long feasibility studies are necessary. Such studies are in particular conducted by the European Commission within the BCR and its successor research programmes. The stabilisation of the material is often one of the major objectives of these studies.

4.2.1.4. Limits to representativeness

Besides the fact that it is economically unrealistic to prepare materials for all single purposes, it is also impossible to produce materials which are fully similar to natural samples. It is, for example, impossible to produce a CRM of 'fresh strawberries' as was requested once to BCR! In environmental monitoring, it has to be accepted that natural samples vary in composition and the analytical methods have to be robust enough to properly tackle a larger variety of samples. Therefore, the analyst has to accept compromises. CRMs will be fully representative only for part of the samples treated in the laboratory. Compromises can be of a various nature: on matrix similarity, content of substances, physical status etc. It is a choice to be made in consideration of the analytical impact these changes will induce when using the material. It is better to select a material that poses similar problems or more difficulties than the most difficult samples tackled by the method.

4.2.1.5. Artificial matrix materials

It is possible to produce artificial matrix materials [12]. Such materials can be prepared on a mass basis by weighing all components both to mimic the matrix composition and the content of trace elements or trace organic substances. The materials could help to have matrix materials available for which the exact contents and composition are known. As a consequence it would be, in theory, possible to certify them on a mass basis and validate methods with highly traceable materials. In organic trace analysis this would circumvent the unknown extraction step. In reality, this is much more difficult to achieve than can be expected. The real matrix composition of many materials is unknown — in particular for environment samples. The physico-chemical status of the various substances depends on the history of the material. Therefore, various natural samples of expected similar composition are different in behaviour. In addition, when preparing mixtures of solid components, losses cannot be excluded and unfortunately are not quantifiable. Attempts have been made where losses were demonstrated but not quantified [12]. Therefore, materials certified for matrix composition and analyte content on a mass basis do not yet exist or are not of real use for method validation by routine laboratories. They may be of interest for laboratories active in the field of fundamental research in chemical metrology where smaller quantities of material are handled.

4.2.1.6. Fresh materials

For several years, environmental analytical chemists have criticised producers because the CRMs available concern only dried materials. This situation was created, historically, by the fact that all CRM producers gained their experience in more

industrially-oriented materials, raw or manufactured products. In the 1970s some natural plant and animal tissue materials appeared in the NBS catalogue (now NIST). All these materials were certified for trace elements and the fact that they were in a dry form did not matter so much. Generations of analytical chemists have used the oyster tissue, olive leaves and a few other NBS materials to validate their methods. With the rapid increase and diversity of organic trace analysis the need for new materials appeared. The certification of RMs for organic trace substances required a new approach, based on interlaboratory studies, because no definitive methods exist. The preparation, the stabilisation and all other studies performed around CRMs had to be adapted. In a first approach the producers relied on stabilisation by drying the materials, like for inorganic analysis. Unfortunately, one of the major difficult steps in organic analysis is the extraction. Dried materials do not behave in the same manner as fresh ones. Therefore, they are of little help to validate the extraction procedure and materials have to be wetted before use. Organo-metallic and inorganic speciation measurements have strengthened this gap. Since the early 1990s, the European Commission (BCR) has in its various calls for proposals of research received more and more projects dealing with fresh materials for organic trace analysis. The first CRM issued from this research arrived on the market in 1992 — BCR-CRM 349 and 350, PCB in fish liver oil [13], followed by pesticides in pork fat — BCR-CRM 430 [7]. Some years later, OCP in fish liver oil BCR-CRM 598 [14], and fresh mussel tissue certified for PCB (BCR-CRM 682) were attempted and others are under study e.g. PAH and their metabolites in fish oil, PCB and planar CB in fish tissue [15]. All these materials are stabilised by addition of preservative substances (e.g. BHT) and are to be used rapidly after opening of the tin cans in which they are stored. This 'new generation' of CRMs seems particularly interesting for environment, food and biomedical quality control purposes.

4.2.1.7. Series of materials

As mentioned above, the matrix often has an important influence on the performance of the analytical methods. In recognition of this situation, and to help analytical chemists as much as possible — in particular in their efforts to control the quality of the methods applied for regulatory purposes — series of materials have been developed by several producers. This is the case for plant materials, soils and sludge, sediments, and animal tissues. The series cover differing matrices but also different contents in substances. Table 5.2 in chapter 5 of this book gives several examples of such series of materials available from major suppliers.

4.2.2. Stabilisation of the material

Reference materials find their utility because the analyst can use them when he wants. Therefore, they must be stable. CRMs have a guaranteed — certified — content of a substance; the producer must guarantee the stability of this substance and of the matrix. RMs for intralaboratory and interlaboratory studies must also be stable. As already mentioned above, stabilisation of the material must be done in such a way that it does not affect the representativeness of the material. In the food/feed control field, as well as in the

field of environmental monitoring, materials are very diverse and complex in composition and are usually naturally unstable. Materials can be altered by three types of activities:

- chemical activity
- physical effects
- biological activity

4.2.2.1. Physical and chemical effects

Physical influences are usually due to light or any other radiation, temperature, and/or gravity. These can be easily avoided by proper storage and transport conditions, e.g. using opaque matter, brown glass bottles or other UV-tight material, stored at adequate temperatures, shaking before use. Liquids and gases can suffer instability due to diffusion of substances through or adsorption on container surfaces.

Chemical activity can occur whenever reactions are possible. Usually, with solid and dry forms these reactions do not happen or take place only slowly. Here too low temperatures decrease the risk. Natural food samples such as freeze-dried milk or fruit juice can be subject to Maillard condensations.

4.2.2.2. Microbial activity

Microbial activities are by far the most difficult factor of instability when handling natural products. All products have an incurred microbial activity (e.g. soils, sediments, sludge) or represent perfect culture media for microbes (e.g. food, feed, human, animal or plant tissues). Bacteria, viruses, moulds, fungi, parasites etc. may also present a risk for the health of the producer or the end-user of the material. In order to eliminate or control microbial activity two possibilities exist:

- kill the microbes
- block the development of the microbes temporarily.

4.2.2.2.1. Destruction of micro-organisms

It has to be stated here that the microbes may represent first a factor of risk for the producer, more rarely the end-user. Materials of human origin and in particular human body fluids have to be treated in a secure manner. Risk of contamination by AIDS, hepatitis B or C occurs mainly at the preparation phase; these organisms are fortunately very fragile and their long-term pathogenicity is negligible. Preparation operations with such materials require all necessary care. These safety conditions have to be known and adequate facilities must be made available by the manufacturing laboratory. Fortunately, their handling requires particular authorisations (in many countries, e.g., a license to handle human blood or tissues is required). These restrictions are also a good guarantee that the material will be safe for the end user. Some infectious organisms are very resistant and necessitate more drastic action to destroy them or to eliminate their pathogenic properties e.g. hepatitis A, cysts, spores etc.

In general, when preparing natural materials that present particular difficulties because of their microbial activity (degradation or contamination risk), it is better to consult food (industry) or biological specialists on the ways of preservation of materials.

Heat sterilisation: Heat preservation is the cheapest procedure. It can only be applied when the substance to be monitored is not affected by the heat treatment. The more the material is contaminated by microbes, the more drastic must be the treatment. Soil and sludge (which might contain hepatitis A viruses which are known to be resistant) must be heated for long periods, typically several hours at temperatures over 100°C. Repeated treatment cycles (heating, cooling, mixing) are preferred to one long heating period. High temperatures will also eliminate volatile compounds. Heating in a dry atmosphere is less effective against microbes than steam heating. Microbes able to resist in a spore form are more resistant and require longer treatments. High temperatures must be avoided when the final goal is to measure organic or organo-metallic substances as they may be degraded or lost. In such circumstances, pasteurisation-like procedures can be applied. Heating at temperatures around 80°C for some minutes, cooling down and repeating heating after a while, will finally kill all forms of microbes including spores. Vegetative forms of organism are killed when heating; cooling will allow spores to transform into a vegetative form, the next heating cycle will kill these newly active particles. Such cycles must be repeated several times. Such mild heating procedures can still be too strong for some materials but they may also be insufficient to sterilise others.

Irradiation: Natural samples can also be gamma-irradiated using a ^{60}Co source. The irradiation dose necessary to reach a full sterility of the material will depend on the matrix ability to stop the gamma-rays. Doses of at least 15 kGy are necessary for solid materials like soil and sludge. Irradiation is not harmless to substances present in the material. Therefore, it can affect the representativeness of a (C)RM. Tuinstra et al. have studied the influence of gamma-irradiation on the content of OCP in animal feed [12]. As shown in Table 4.1, up to 50% of the content of lindane is already destroyed by a 5 kGy irradiation. As the material was foreseen to validate methods at a specific level of OCP content, a loss of 50% was no longer acceptable. In addition, irradiation might produce reaction compounds which are not naturally present in the material and which could affect the analytical method (e.g. apparition of 'ghost' peaks in chromatograms, interferences).

Antiseptics: An easy way to destroy microbes is to add an antiseptic to the material. This can only be done if this antiseptic can be added in a sufficient amount and does not affect the analyte or the analytical behaviour of the material unduly. Such a stabiliser, butylated hydroxytoluene (BHT), has been used by BCR several times in the production of fish oil and fresh mussel tissue materials certified for PCBs [13–15].

4.2.2.2. *Blocking microbial development*

When microbes cannot be destroyed because all available methods affect the material too much or when the microbes themselves have to be measured, they must be stabilised by other means. Microbes have been stabilised in microbiological BCR-CRMs by spray drying them within cow's milk [16]. Only few strains of bacteria supported the treatment and the resulting materials can be stored over several years without any decrease nor increase in the colony-forming particles they contain [16–21]. This demonstrates that an RM can be stabilised for a very long time by just blocking the microbial activity in a dry matrix without killing them. Microbes can also be blocked in their activity by putting them into a hostile environment. Two procedures are possible: increasing the

TABLE 4.1

DEGRADATION OF ORGANOCHLORINE PESTICIDES IN PORK FAT BY -
IRRADIATION (^{60}Co SOURCE; IRRADIATION DOSE = 0, 2.5 AND 5.0 KGy).

The data have been normalised to the content determined on five test portions which were not irradiated (base = 100). the data given are the normalised means of five determinations and the corresponding standard deviations (after [53]). up to 50% losses are noticed for one substance already at 2.5 kGy

Pesticide	Irradiation dose		
	0	2.5 kGy	5.0 kGy
HCB	100±4.4	101±5	104±2
(α)-HCH	100±2.6	99±7	95±2
(β)-HCH	100±7.8	90±8	101±6
(γ)-HCH (lindane)	100±3.1	54±4	52±3
Heptachlor	100±4.6	110±3	111±2
Aldrin	100±2.6	108±3	106±2
(β)-Heptachlor	100±6.5	103±3	99±3
(γ)-Chlordane	100±2.3	97±4	102±3
(α)-Endosulfan	100±3.6	79±3	76±3
p,p'-DDE	100±4.2	94±4	103±3
p,p'-TDE	100±3.4	104±5	99±4
o,p'-DDT	100±3.6	98±4	102±2
p,p'-DDT	100±2.4	84±5	85±2
Dieldrin	100±1.4	93±3	95±5
Endrin	100±2.7	102±7	90±2

ionic strength of the matrix or eliminating water (both procedures have long been used for food preservation). Eliminating water is the most common stabilisation method. Removal of water can be obtained by simple heating (spray-drying) or, for very heat sensitive materials, by freeze-drying. Lyophilisation is an expensive technique which, for large quantities, necessitates equipment existing mainly in the food or pharmaceutical industry. Therefore, it is reserved for materials with high economic or analytical added value [16,23–26]. Figure 4.1 shows an example of a freeze-drying chamber with inside individual protection doors. A material with a water content of less than 5% (mass fraction) should present no risk of bacterial growth nor chemical reactions. This is true only as long as the vial in which it is stored remains closed. When the operator opens it for sub-sampling and allows moisture to enter the stability may be lost. In some cases, microbes can be eliminated by micro-filtration e.g. serum, beverages, water.

4.2.2.3. When to stabilise the material

The material can be stabilised at two stages: during or after preparation. When the stabilisation affects the homogeneity or the analytical behaviour of the material, the

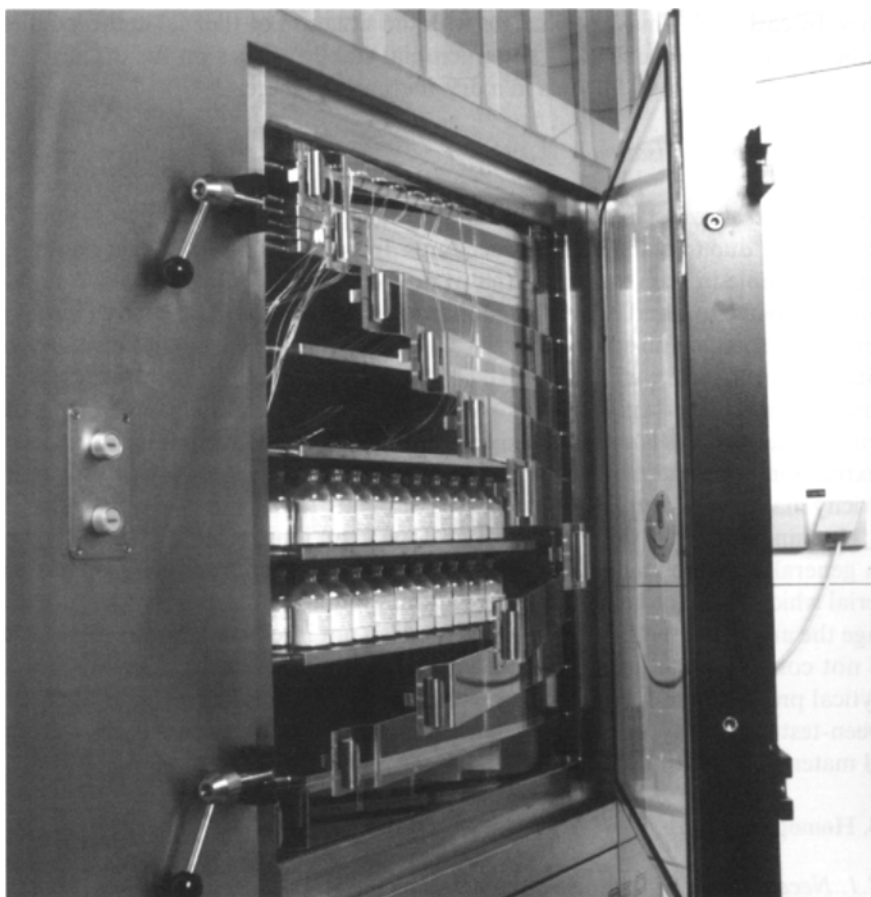


Fig. 4.1. Freeze drying chamber with inside individual protection doors (courtesy of IRMM-JRC, Geel, Belgium).

treatment must be performed on the bulk, before final homogenisation and splitting into vials. For dangerous materials (infectious or matrices containing toxic substances) it is also important to reduce the risk at the beginning of handling. When stabilisation must eliminate microbes it is important that no contamination occurs after the sterilisation. This can be achieved by working in closed cupboards, glove boxes, laminar air-flows and/or under UV light.

If the stabilisation does not affect the analytical behaviour of the material and if handling is not dangerous, it is preferable to apply the stabilisation on the finished material or just before packaging. In particular removal of water will remain more efficient when performed just before packaging. The most ideal stabilisation against microbes is gamma-irradiation as it can be performed on the packed material through the container walls. This is not valid for materials certified for organic or organo-metallic substances as the destruction rate of the compounds depends on the dose

received by each vial. This dose changes with the situation of the vial in the irradiation chamber or tray. When chemical and microbial stability relies on the dryness of the material it is preferable to fill vials in a dry, inert atmosphere.

4.2.2.4. Limits to stabilisation

As discussed above, stabilisation can induce changes in material composition. Other effects may be due to stabilisation procedures. In particular, a low water content can affect the sample in such a manner that it no longer behaves like natural samples or becomes difficult to handle. Very small particle size increases the contact surface of solvents or reagents with solid materials. Consequently, the extraction or digestion is facilitated. The opposite effect can also be obtained if, in addition to drastic grinding, the material is too dry e.g. water content of less than 1%. In such cases, the surface activity of the particles is so increased that the contact of the solvent is impossible and the extraction is hampered. With very fine ground materials in a very dry stage, static electricity makes sub-sampling from a bottle difficult. This is the case with BCR-CRM 397, 'Human Hair'. Sub-sampling must be done in an ionised atmosphere [27].

In general limits to stabilisation appear when the procedure to be used leads to a material which does not behave at all as natural samples. Materials that require one to change the analytical method to be tested are also not interesting for the analyst. This does not concern materials which need a preparation step before they undergo the analytical procedure as long as this preparation step does not add significantly to the between-test variability. For example a simple dissolution on a mass basis, wetting of dried materials etc. can be acceptable.

4.2.3. Homogenisation

4.2.3.1. Necessity for homogeneous samples

(C)RMs are used to assess the performance of analytical methods or to calibrate measurements. They help to reach and to demonstrate comparability between methods and laboratories. They can also be a way of linking results of measurements to recognised references and possibly, as recommended by ISO 25 [28], to the S.I. units. To do so (C)RMs have to be used or circulated over longer periods of time (long term reproducibility) and over space (between laboratories). Chemical measurements are in the majority of cases destructive: the test sample at the end of the measurement is altered in its physico-chemical status and cannot be measured again. To fulfil the time and space objective, the materials have to be produced in several units. All units have to be fully identical. The availability of large numbers of identical test samples requires the preparation of large quantities of materials. This large batch of material will be homogeneous so that all the sub-samples are identical with respect to the property to be measured and assessed. In the case of certified reference materials the certified values must be backed-up by a demonstration of the homogeneity. Therefore, the homogeneity should be assessed in relation to the measurand and the size of the test sample (sample intake). This condition is sometimes difficult to fulfill.

In chemical measurements three major types of materials are analysed: gases, liquids and solids. All intermediate mixtures of phases can be encountered e.g. aerosols, emulsions. All pose specific difficulties in the preparation and in particular for homogeneity.

4.2.3.2. *Homogenisation of fluids*

Mixing gases or miscible liquids or dissolving a soluble solid into a solvent and keeping the mixture homogeneous is 'easy', at least when dealing with laboratory scale amounts e.g. grams or litres. When very large quantities of materials must be handled e.g. tons, cubic-meters, then industrial scale equipment and procedures must be applied. Mixing of gases can only be performed in highly qualified laboratories with specific skills and equipment. In general, single-phase solutions are easier to homogenise than complex — multiphase — mixtures. The main difficulty is to have available a sufficiently large container to perform the mixing in one batch. The classical principle of successive dilutions e.g. starting from a concentrated solution and diluting it stepwise with a pure solvent up to the desired concentration, while assuring mixing, is always applicable and is a safe way of handling.

The homogeneity of solutions and gas mixtures is also easy to maintain or to reconstitute. The vast majority of calibration CRMs are pure solid substances or solutions. The only difficulty encountered by the manufacturer is the availability of adapted preparation 'tanks' in which large volumes of solutions can be handled, with sufficient stirring capabilities, and without any risk of contamination. Emulsions need to be stabilised through adequate addition of emulsifiers or dispersion products. Such additives may pose the problem of representativeness of the final material.

The preparation of mixtures of solids e.g. for producing metallic targets or powder pellets, relies mainly on the availability of proper balances and mixers.

4.2.3.3. *Homogenisation of solids*

Multiple phases: Natural or artificial (manufactured) solids can be composed in several phases. The quality of the dispersion of these phases one in the other will allow the analyst to benefit from similar test samples within the entire batch of material. Material sciences teach us that the degree of homogeneity of the mixture will depend on the nature of the mixed phases and, in particular, the size of the solid particles, the particle size distribution and the respective density. The more similar the density and the particle size, the better is the achieved homogeneity. To achieve similar test samples, even for very small particle sizes, requires a narrow particle size distribution. Muntau et al. [29–30], Griepink et al. [31] and Pauwels et al. [32] have studied the relation between particle size and minimum sample intake. They have demonstrated that a major factor for homogeneity of small sample intakes is the low particle size of the solid material and their size distribution. This aspect will be discussed in more details in the section on homogeneity testing.

Solid materials are composed of various dispersed phases, e.g. alumino-silicates, oxides, organic matter in soils, fat and fibres in tissues of animal organs etc. Within

such a matrix many trace elements or substances are unequally dispersed. Various matrix components present various abilities to absorb or adsorb trace substances, e.g. trace organic substances having a tendency to be concentrated in the organic phase of a soil, sediment or sludge, or in fatty tissues of living organisms.

Grinding techniques and sieving: Solids are usually first sorted to remove unnecessary large pieces or unwanted material, e.g. fat from muscle tissue, large stones or pieces of roots from soil or sediments. It facilitates further treatment. Materials are also air-dried and sieved before any other treatment. Grinding using all classical means is the next step of the homogenisation. Grinding with an Agate or Platinum mortar for small quantities of minerals achieve a sufficiently fine powder for laboratory RMs. For plants or animal organs and for large heterogeneous materials ball mills, knife crushers etc. are sufficient for first grinding. To achieve finer particles, more sophisticated apparatus is necessary, in particular for the production of large batches of CRMs [33]. The most practical instrument is the jet-milling apparatus, which also allows the selection of particle size within narrow ranges. The jet-mill also allows one to work in a closed vessel that avoids exposure of manpower to toxic substances (details are given in Table 4.2). For all grinders and crushers it is necessary to verify that the material (metals) constituting the working vessel is not to be measured in the material. The risk of contamination with metallic particles is high (e.g. Ni, Co, V, Fe, Mo of stainless steel). So-called spot contamination may forbid the use of the material for microanalysis. Figures 4.2a–c show different grinding/milling devices, namely a ball mill with PTFE jars (Figure 4.2a), a cryo-ball milling procedure (Figure 4.2b), and a jet-mill device (Figure 4.2c).

Between grinding steps, it is always advisable to perform a sieving step. This allows one to eliminate remaining large particle phases or discard the powdered portion, which is already fine enough. It also gives an overview of the particle size distribution. This property is best checked by particle size determination, which is nowadays possible with low cost laboratory-scale instruments. An example of a sieving machine is shown in Figure 4.3.

When properly ground and mixed, the material can be brought to a stage where very small portions are representative of the whole batch of material. When no difference between these small portions can be detected the material is called homogeneous for the studied test portion or sample intake. The smaller this portion is, the better the homogeneity.

Splitting or riffing: When properly homogenised the material has to be split into portions that will be filled into an appropriate container. When the grinding/homogenisation has been performed in several batches, e.g. because the preparation instruments had too small a capacity, riffing procedures have to be applied with cross mixing (coal CRM). The final splitting into portions for filling can also be performed by a riffler. In Table 4.3 a riffing plan for a BCR-CRM (PAH and PCP in wood) is given. Figure 4.4 shows a laboratory-scale riffing machine used for coal materials.

During the filling procedure, mixing of the remaining bulk must be done to avoid segregation of denser particles in the mixing drum (see example of Turbula mixer in Figure 4.5). Figure 4.6 shows an example of cone mixer and filling device.

Finally, as shown in Figure 4.7, stabilisation of the material may be necessary at various stages of the procedure. The best choice will depend on the type of material, size of batch and equipment available.

TABLE 4.2

EXAMPLES OF GRINDING MACHINES FOR THE PREPARATION OF SOLID MATRIX CRMS. SIMILAR TABLES COULD BE PREPARED FOR MIXING, SIEVING AND PARTICLE SIZE DETERMINATION DEVICES. MORE INFORMATION CAN BE FOUND FROM SUPPLIERS AND IN REF. [54-57]

Type of machine	Function	Capacity	Type of material or matrix	Remark
Manual mortar	Crushing and mixing	Some 100 g	Solids, pastes	Agate or platinum
Mortar grinder	As above but automatic	Up to 250 ml	As above manual	As above
Jaw crusher	Rough crushing	> 100 kg/h for large scale machines	Medium hard, brittle, hard solids	Approx. 1 mm
Teflon Jaw crusher	Coarse	5-15 kg/h	Frozen animal tissues []	To avoid metal contamination
Rotor beater mill	Precrushing to fine grinding	Up to 300 kg/h	Soft to medium hard materials	Down to < 100 µm
B.M. agate balls B.M. corundum B.M. ZrO ₂ B.M. Cr steel B.M. Cr-Ni steel B.M. WC/Co B.M. Teflon B.M. polyamide	Balls and material of the mill must be adapted to the type of matrix and analyte (spot contamination)	From less than 1 ml to 500 ml or 500 g for laboratory size, up to several kg for industrial scale	Soft samples Fibrous Fibrous + abrasive Medium-hard + brittle Hard + brittle Hard + abrasive Frozen or freeze-dried plant or animal tissues [] Soft + brittle	Particles of 1 mm to 1 µm. Can work under inert atmosphere. For solids, suspensions, colloids, emulsions
Rotor mill	Grinding	Continuous feeding	Soft to medium hard and fibres	Risk of over heating
Centrifugal mill	As for B.M.	Low capacity	As above B.M.	Less than 1 µm particles
Vibrating mill	Rapid grinding with vibration disks	250 ml max.	Geological, metallurgy, hard materials	WC/Co, agate, Cr steel disks, particles of less than 0.1 mm.
Disk mill	Continuous, rough grinding	150 kg/hour	Hard solids	0.1 mm minimum
High speed rotor mill	Cutting/grinding of soft materials	Continuous feeding	No hard materials	Stainless steel mat cause spot contamination
Cutting mill	Stainless steel knives	Continuous feeding	Soft to medium hard material	Minimum 250 µm particles
Jet-mill	Fine and soft grinder working under air jet	4-6 kg/h	All types of materials	Protected from external contamination [] down to few µm particles
Impact-mill	Similar to jet-mill	As above jet-mill	As above	Particle size selection possible

B.M.: ball mill or planetary mill



Fig. 4.2a. Ball-mill with PTFE Jars (courtesy of IRMM-JRC, Geel, Belgium).

During the filling procedure and for each bottled batch a sample should be set aside for the later verification of the homogeneity.

4.2.4. Packaging and storage

4.2.4.1. Packaging material and vial

The vial in which the material will be stored, together with the storage conditions, will guarantee that the preparation work performed will remain unchanged. The packaging material used must be inert to the matrix and the substances of interest. Interaction between material and vial mainly occurs with liquids or gases. Gases may diffuse into or through the walls, liquids may favour adsorption or absorption phenomena. In extreme cases, the vial material may contaminate the reference material e.g. additives in plastics, trace elements from glass or metals etc.



Fig. 4.2b. Cryo-ball milling. (Courtesy of IRMM-JRC, Geel, Belgium).

The packaging material must also guarantee that the material is isolated from the outside. It must be made of non-porous matter. The vial must be closed with an adequate system. Protection from light and in particular UV is obtained with brown glass or opaque matter. The head-space above the material should be minimal to avoid evaporation and condensation phenomena in the vial. This could induce risk of heterogeneity of the material.

Contamination by laboratory atmosphere, e.g. volatile substances, dust, or humidity, is circumvented by the use of glass, quartz (very expensive!) [34] or metals but the latter are also heavy. Metal cans must often be lined with a polymer film in order to avoid oxidation phenomena [15]. If used as ampoules, glass and quartz can be soldered; in this case they can only be used once or must be soldered again after sub-sampling. Care must be given to the fact that glass ages, and that the soldered part can become loose. When soldering again after a first use, the operator must take care to not affect the material (solvent, substances) stored in the vial. Metal cans should be soldered. Glass

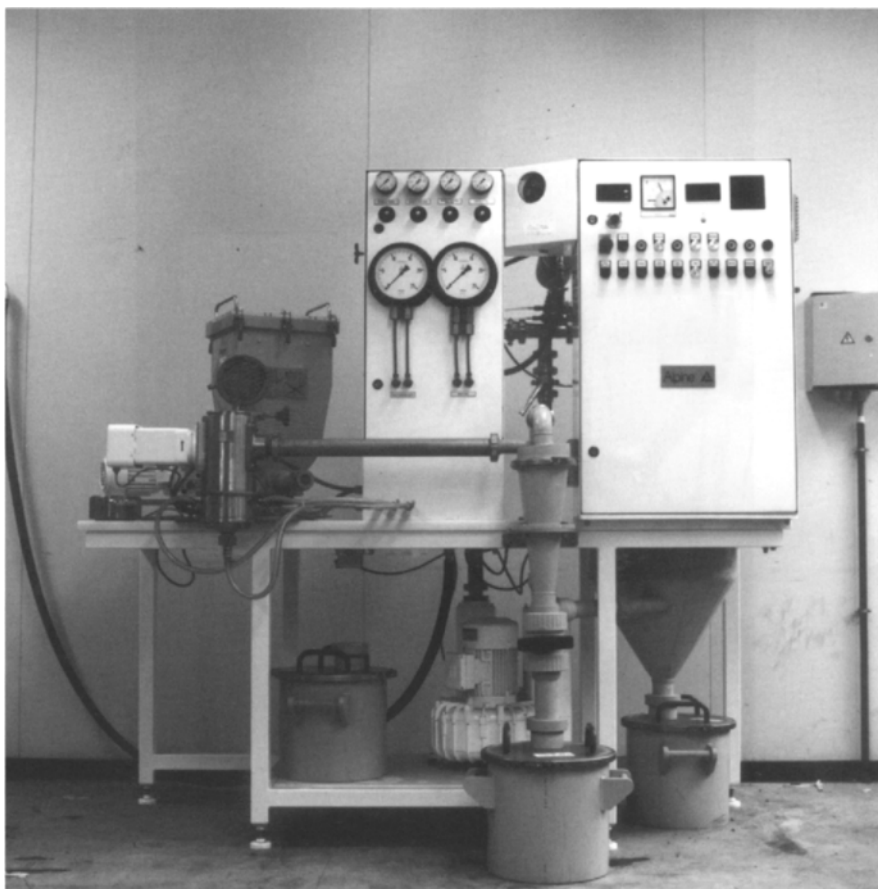


Fig. 4.2c. Jet-mill. (Courtesy of IRMM-JRC, Geel, Belgium).

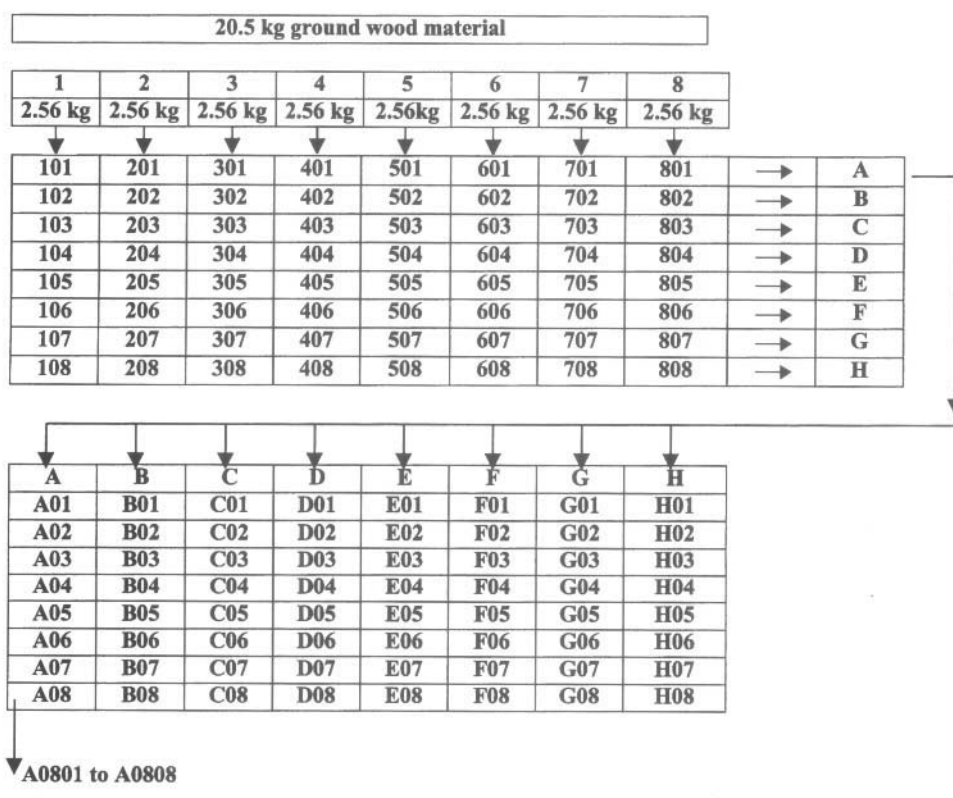
and polymer bottles are usually closed with a screw cap. Tightness is assured by an insert made of an inert polymer: polythene, PTFE, PVC etc for RMs certified for inorganic substances, or lined with aluminium for RMs certified for trace organic substances. Screws and inserts must be of high quality so that they can be opened and closed several times without losing tightness. When bottles are used for liquids it is advisable to reinforce the closing system with an aluminium cap setting (penicillin type of bottles). The end-user should preferably reset a fresh cap after opening. Packaging and in particular closing systems should be of the highest possible quality as it is for all high added value products.

Polymers are not always tight to liquids. Water can slowly perspire through the walls. Such a situation has happened and has been circumvented with BCR-CRM 398 and 399 — trace and minor elements in fresh water — by storing the polythene bottle in a metal can in which the atmosphere is rapidly saturated by vapour and stops perspiration [35]. In this way the CRM is available over years. The best polymers

TABLE 4.3

RIFFLING SCHEME FOR THE HOMOGENISATION OF WOOD SAMPLES FOR THE DETERMINATION OF PCP AND PAH [58,59]

20.5 KG FINE GROUND WOOD MATERIAL ARE SPLIT INTO 8 PORTIONS OF 2.56 KG (01 TO 08), EACH IS RE-HOMOGENISED INDIVIDUALLY AND SUB-SPLIT INTO 8 PORTIONS (×01 TO ×08). THE VARIOUS 01 PORTIONS ARE POOLED AND HOMOGENISED INTO A PORTION A OF 2.56 KG. SIMILARLY FOR THE SUB-PORTIONS 02 TO 08. THE OBTAINED SETS A TO H ARE EACH SPLIT INTO 8 SUB-PORTIONS OF 320 G (A01 TO A08 UP TO H01-H08, 64 SUB-PORTIONS IN TOTAL). EACH SUB-PORTION IS SPLIT INTO EIGHT FINAL PORTIONS OF 40 G. A TOTAL OF 512 SAMPLES ARE PRODUCED.



for water and aqueous solutions are high-density polythene, PTFE. Organic solutions are usually distributed in metal or glass vials. For some solid materials, e.g. seed, soils, or other agriculture CRMs, BCR has introduced aluminium foil sachets lined with a polymer. They are light and handy but sometimes criticised as they cannot be re-closed after a first use. Polymers are also used nowadays to protect metal disks to avoid oxidation.

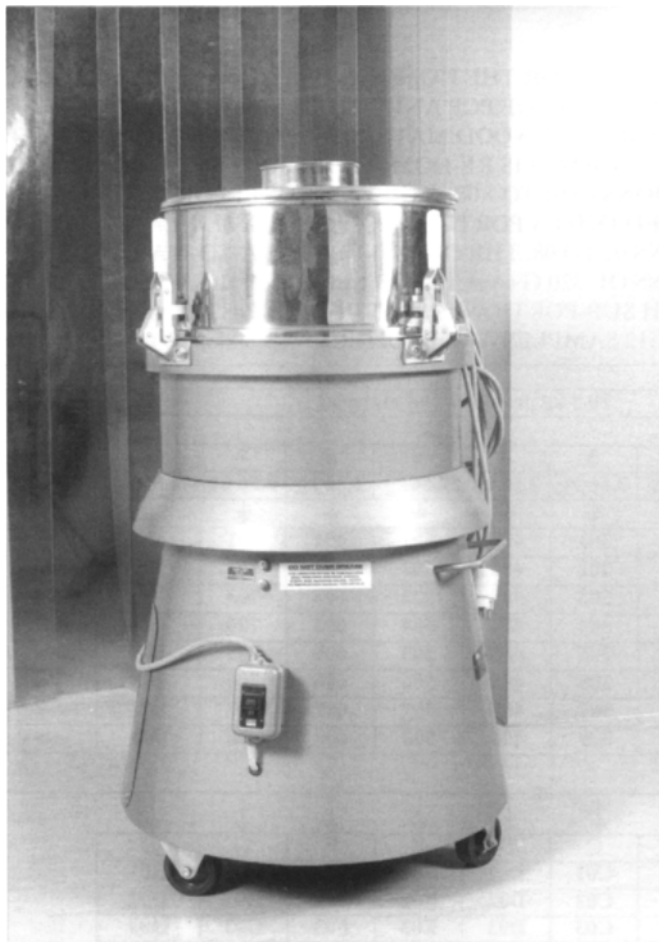


Fig. 4.3. Sieving machine (Type Russel). (Courtesy of IRMM-JRC, Geel, Belgium).

The most difficult situations are encountered with living organisms or substances for which activities must be measured. BCR has developed storage in gelatine capsules for microbiological RMs and CRMs [16].

These are only general remarks. Each individual situation has to be studied for the best suited packaging material, taking into consideration storage duration, usage, type of matrix, and substances to be measured. Preliminary studies are necessary to decide the most appropriate and economically sustainable solution. It also underlines the necessity to use a multidisciplinary approach to these feasibility studies, e.g. specialists in various disciplines. Several examples are given later in this book mainly taken from the field of environmental monitoring. Table 4.4 shows examples of packaging used by BCR.



Fig. 4.4. Riffing machine. Laboratory scale from SBN (The Netherlands).



Fig. 4.5. Turbula mixer. (Courtesy of IRMM-JRC, Geel, Belgium).

4.2.4.2. Storage temperature

The conditions for the long term storage of the (C)RM have to be determined as a conclusion from the preliminary feasibility studies or from the study of the stability. Conservative measures can be applied in case stability cannot be fully guaranteed (e.g. storage at a temperature lower than the lowest temperature for which no instability could be found). Such conservative measures must remain economically acceptable.

4.2.4.3. Safety and information

In addition to the protection of the material itself, the packaging vial has two other roles:

- protecting the users
- information to the users



Fig. 4.6. Cone mixer with riffling device. (Courtesy of IRMM-JRC, Geel, Belgium).

These two roles are important for materials that might harm the health of users and all those who have to handle them, e.g. carriers, sellers, custom agents. The storage container must be resistant (glass breaks easily!) and tight enough to avoid any release of material in case of accident during handling. It is particularly important for toxic materials, microbial RMs, fresh human or animal tissues, and for plant materials like seeds in countries where import of such items is regulated. Usually, the storage conditions to secure the material's integrity described above are sufficient for the protection of the environment. The information aspects on security and safety are then carried by the label stuck on the container and are on the accompanying information e.g. certificate. Regulations exist in various countries and have been issued by transport regulators (e.g. IATA for air freight) on what and how safety and security information has to be given. Some safety regulations imply that the toxic materials are transported in special containers requiring an *a priori* acceptance agreement (e.g. nuclear materials, dioxins).

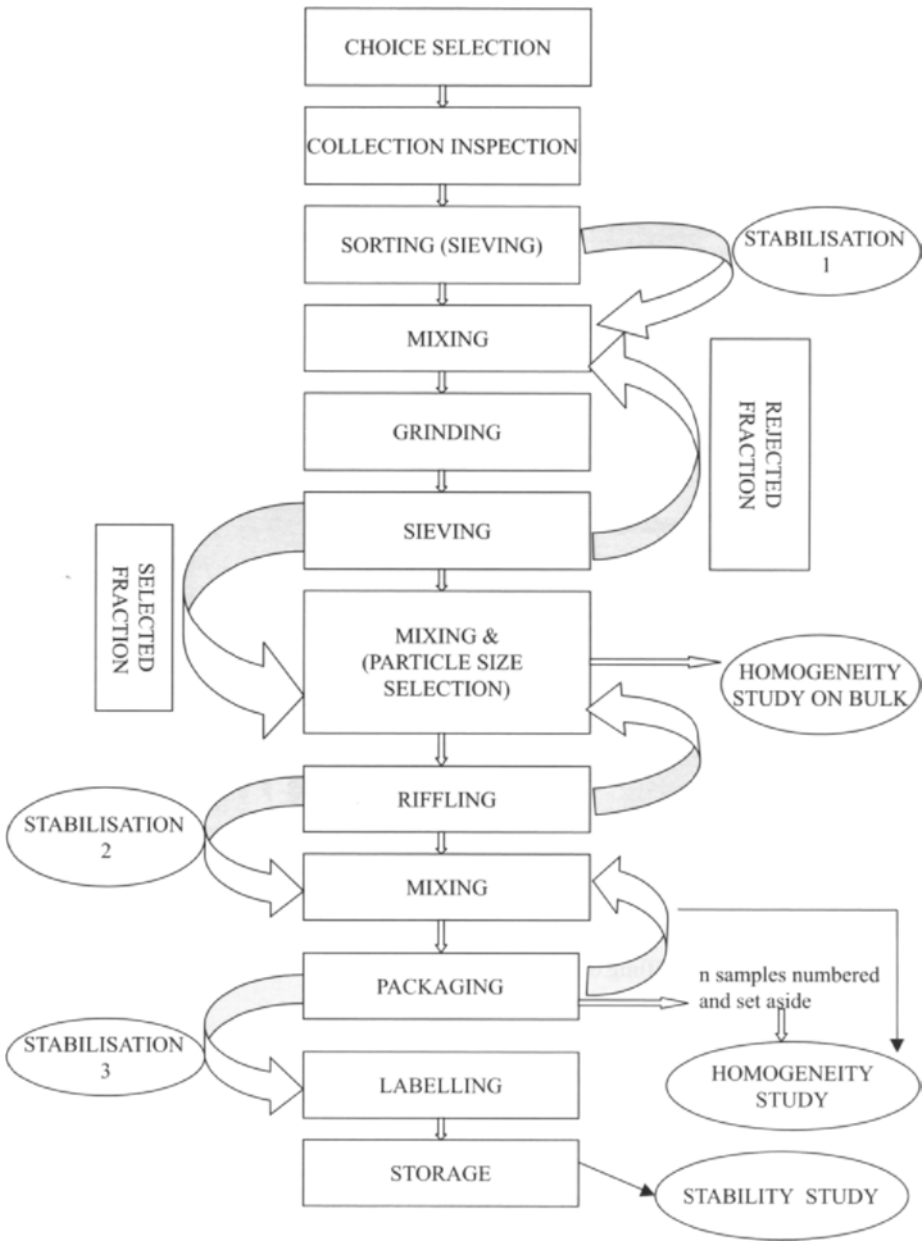


Fig. 4.7. Classical scheme for the preparation of matrix reference materials. Stabilisation of the material can be performed at various stages of the procedure and it can also be repeated at various stages. During the preparation a lack of homogeneity or stability may require recommencing a step of the treatment. For the study of the homogeneity test samples must be selected in accordance with the preparation. When packaging is split into batches a sample of each batch must be set aside for the homogeneity study.

TABLE 4.4

EXAMPLES OF PACKAGING OF BCR CRMS

Type of CRM	Packaging
Pure PAH or PCB compounds	Amber vials containing 10 to 100 mg of powdered material
Soils / sludge / sediment	Glass bottles containing 25 to 70 g
Aquatic/terrestrial plants	Glass bottles containing 20 to 30 g
Biological materials (mussel, fish, etc.)	Glass bottles containing 5 to 15 g
Fly ash	Amber glass bottles (dioxins)
	Ampoules (trace elements)
Urban dust	Glass bottles containing ca. 15 g
Freshwater	Polypropylene bottles (major elements)
	Glass ampoules (nitrate)
Artificial rainwater	Sealed quartz ampoules
Artificial groundwater	Glass ampoules (major elements)
Natural groundwater	Brown glass ampoules (bromide)
	Polyethylene bottles (trace elements)
Seawater / estuarine water	Polyethylene bottles (trace elements)
	Glass bottles (mercury)
Lyophilised solution	Glass vial containing powdered material (Cr-species, pesticides)
Pure solution	Glass bottles (Arsenobetaine)
Fish oil	Sealed glass ampoules
Waste mineral oil	Glass ampoules
Microbes in milk powder	Gelatine capsules

4.2.5. Size of batch

The size of the batch of material will depend on the objective assessed for the material. Small batches are sufficient for intralaboratory first level control e.g. litre(s) of liquids, some grams of solids etc. For proficiency testing, depending on the number of participants, the batch may be larger, e.g. some kilograms or several litres. In general, the production of CRMs requires the production of several hundred sets, in turn requiring hundreds of kilograms or tons of solid materials or several cubic meters of liquids. For CRM production, as the investment is important, a preliminary market study may be necessary to estimate batch size as it also helps to establish a price affordable by customers. It can also avoid unnecessary investment in machines or storage facilities or repeated production of the same material.

4.2.6. Transport

If not produced in the laboratory itself where it will be used, the CRM must be delivered properly to the end-user. Additional packaging precautions and transport safety aspects need to be considered. During transport, the main risk is due to shock

and drastic changes in temperature. (C)RMs stored in simple glass containers can be dangerous as glass is fragile. They should be at least sealed into a polymer bag. They must be packed in shock-resistant parcels. Those suffering temperature increase must be handled in dry-ice, or thermostatic parcels. In that case they should preferably include temperature indicators which reveal temperature increase during transport. Fast delivery is the safest way of bringing such materials to the user. In general the duration for delivery should remain at a minimum. Feedback to the producer on the date and condition of receipt by the end-user should be foreseen for 'difficult' materials (reply forms can be sent with the parcel).

4.3. STUDY OF THE HOMOGENEITY

4.3.1. Principle

When a material is used as a reference for chemical measurements it must be guaranteed that all sub-samples taken for this purpose are identical. As the preparation, and in particular the stabilisation of a material, is a complex succession of treatments, several sources of mistakes (local alterations, contamination, etc) can modify the composition during the handling or from one set to the other. Therefore, the demonstration of the homogeneity of the material is essential for the validity of e.g. the certified or stated value of a parameter in the (C)RM. To assess that a material is homogeneous the analyst usually tries to detect heterogeneity (inhomogeneity). For this he must use a measurement method. As for the preparation, the test for homogeneity is the most difficult for solid materials in which traces of substances are to be measured. The following discussions relate mainly if not only to solid materials or multiple phase materials.

As discussed in the previous chapter a preparation procedure leads to several sets of samples, often produced in batches. It is necessary to assess that no difference exists within each of the sets, between sets and between batches. Consequently, homogeneity testing will try to measure differences between sub-samples within or between vials of materials. As it is not possible to measure all samples produced (unless a nondestructive technique is available) a strategy for the selection of representative samples is necessary. To demonstrate the absence or the existence of differences between samples, it is necessary that the analytical procedure is 'fully' reproducible. If differences between measurements are too large due to the measurement method, inhomogeneity cannot be detected. In order to reveal presence of spot contamination, the measurement must be done on the substance of interest or any other substance known to present exactly the same properties and showing the same behaviour or distribution pattern (tracer).

4.3.1.1. The test method

Differences between test results can be expressed by the coefficient of variation between several measurements obtained on different samples or the confidence interval.

The total uncertainty measured between samples will in fact represent the precision of the measurements U_M plus the contribution due to inhomogeneity U_{INH} :

$$U_T^2 = U_M^2 + U_{INH}^2$$

All analytical procedures have an uncertainty. Usually, this uncertainty is linked to the absolute quantity measured by the detector. It is at a maximum near the limit of detection. After a minimum corresponding to the optimal measurement range it may increase again when saturation of the system starts. The absolute quantity detected depends on the sample intake and the possible concentration steps of the procedure. Ideal methods for homogeneity testing have a very low determination limit and possibly a very wide dynamic linear range. Such methods allow the selection of very small sample intakes as the analyte content is not a limiting factor. Heydorn has demonstrated how INAA can be validated and the uncertainty budget of the applied method can be established so that this method can effectively be used to investigate the minimum sample size of a CRM [36]. Pauwels and Vandecasteele have developed and demonstrated that solid sampling atomic absorption spectrometry with Zeeman background correction (SS-ZAAS) is a method that can perform well in investigating the homogeneity of samples down to some micrograms of sample intake [37,38]. If the amount to be measured in the test sample is far larger than the limit of determination then the analyst can decrease the size of the test sample. If for very small sample sizes the method is still as reproducible, the analyst can try to determine the minimum sample intake for which inhomogeneity can be detected. U_M remains constant but U_T increases. This indicates that U_{INH} increased, signalling the appearance of inhomogeneity. Only with a very precise method will it be possible to detect inhomogeneity in very small samples.

The real difficulties remain in the determination of U_M . It is relatively simple to determine the method uncertainty of 'nondestructive' analysis as repeated measurements can be performed on the same sample [39]. It is far more difficult with destructive methods and in particular in organic trace analysis. In the latter case, all the steps in the procedure rarely allow one to achieve a repeatability with a relative standard deviation of less than several percent. The methods often require a large sample intake as samples of a few milligrams are not easy to handle in extraction systems.

The determination of the uncertainty of the measurement method necessitates the measurement of several samples in the same conditions as in the homogeneity study. This means that U_M must be established on the material itself. U_M is in fact a sum of individual uncertainties generated by each step of the procedure:

$$U_M^2 = U_S^2 + U_E^2 + U_P^2 + U_C^2 + U_D^2 + U_X^2 \dots$$

where S stands for sub-sampling, E for extraction or digestion, P for purification, C for calibration, D for final detection and X for correction factors (e.g. fat content, dry mass etc.). One step may not exist in some methods or additional ones may be present.

When evaluating U_M the analyst has to include the U_S component, which in fact consists in doing a sampling of the material and therefore includes potential heterogeneity or inhomogeneity factors. This often limits the determination of U_M . If the analyst works properly this should not affect too much the determination of U_M as the sub-sampling factor is mainly an uncertainty due to weighing. Various ways exist to

establish the uncertainty of the method e.g. by pretreating a large test sample and splitting the procedure after U_E . Defining U_M just by determining the substance of interest in a pure standard solution is in many cases insufficient (limits U_M to U_C), in particular in organic or organo-metallic trace analysis. An example on how to approach the value of U_M for testing the homogeneity of a material certified for organic trace substances is shown in Table 2.2 of Chapter 2. In Table 4.5, the example of soils shows the limits of the approach. For such materials, the best estimate of the homogeneity comes from information on the preparation procedure such as particles size analysis, not from the measurements.

In general, when trace substances or elements have to be studied, the sensitivity of the method can be a limiting factor. The analyst cannot establish at which level of sample intake inhomogeneity of the material is detectable because the sensitivity of the

TABLE 4.5.

HOMOGENEITY STUDY OF THE CERTIFIED PCDD AND PCDF IN CRM 529 (CLAY SOIL)

The five replicate measurements of the raw extract give an overview of the method's repeatability, the measurements for the within-bottle homogeneity was performed in one batch, the between-bottle homogeneity took several days. it can be noted that the within-bottle cv is often smaller than the cv of the extract measurements, but always smaller than the between-bottle cv. The cv obtained from the certification campaign (12 laboratories each using two bottles) is always much larger than this between-bottle cv.

Substances	Certified value	Raw extract (n=5)	Within-bottle a (n=5)	Within-bottle b (n=5)	Between-bottle (n=20)
CRM 529	CV,%/ p	CV, %	CV, %	CV, %	CV, %
2,3,7,8-TCDD	19 / 12	2.1	1.7	0.7	4.8
1,2,3,7,8-PeCDD	11 / 6	3.2	2.9	1.5	6.9
1,2,3,4,7,8-HxCDD	22 / 9	3.0	3.9	0.8	9.1
1,2,3,6,7,8-HxCDD	23 / 11	2.3	3.4	1.0	9.0
1,2,3,7,8,9-HxCDD	19 / 12	4.5	1.3	1.4	7.7
2,3,7,8-TCDF	18 / 7	6.2	9.9	1.7	10.8
1,2,3,7,8-PeCDF	23 / 8	5.9	2.9	0.8	7.9
2,3,4,7,8-PeCDF	22 / 8	3.4	4.7	1.0	11.0
1,2,3,4,7,8-HxCDF	18 / 9	5.2	4.1	0.3	7.9
1,2,3,6,7,8-HxCDF	21 / 12	7.3	2.8	0.7	8.2
1,2,3,7,8,9-HxCDF	35 / 5	4.5	9.1	0.7	10.4
2,3,4,6,7,8-HxCDF	17 / 12	8.5	4.6	1.0	10.8

n: number of replicates

p: number of data sets accepted for the certification and used to calculate the between laboratory CV.

measurement is insufficient. Therefore, he has to limit the homogeneity study on larger samples or he must extrapolate from the determination of another substance (tracer) for which the method is more sensitive. In general, materials are produced and certified for several parameters. In inorganic trace analysis the analyst has several elements or compounds of similar physico-chemical behaviour on which he can base his homogeneity study. In organo-metallic and organic trace analysis, complex analytical procedures are applied. Often it is difficult to reach repeatability figures better than 5 or even 10% whatever concentrations are measured. Even tracer substances may not always allow better precision e.g. substances of the same chemical family — isomers, congeners etc. Tracer substances or elements must be used with care, as theoretically equally dispersed elements may in fact not behave in the same manner. Pauwels et al. have demonstrated with SS-ZAAS that some elements having even similar concentrations may be dispersed differently. They show nice examples of so-called 'spot' contamination or presence of 'nuggets'. The unequal repartition of elements leads the analyst to define very different minimum sample intakes. For a carefully prepared cod muscle tissue [40] they concluded that the minimum sample intake of the CRM is 12 mg for lead but up to 70 mg for iron. This may come from a less dispersed presence of iron in the material and shows that Fe could not be traced by Pb in this material. Pauwels et al. have also performed a similar study on plastic materials [37]. They have determined that the minimum sample intake for Cd in four different polyethylene materials used by the car industry lies between 13 mg and 1 g. The material with the worst homogeneity presented clearly coloured Cd spots. This demonstrates that families of materials may be very different at the level of homogeneity even if they have been processed similarly. The same group of authors has studied extensively the use of SS-ZAAS for the determination of micro-homogeneity and in particular the determination of the minimum sample intake when nugget repartition is suspected [41]. The availability of such highly sensitive and performing methods for the study of the homogeneity of a CRM is of very high value. Unfortunately, nothing like that exists in organic trace analysis.

4.3.1.2. Conclusion and limits of the study

The total uncertainty U_T will decrease with increasing sample size as the amount to be measured increases and because the possible inhomogeneity disappears. For a certain sample size which exceeds the working range of the method, U_T may again increase as the method runs out of its linear dynamic range (e.g. saturation of detector, overloaded separation or clean-up system etc.). When studying homogeneity, if the uncertainty due to the method (U_M) is very large compared to uncertainty due to inhomogeneity (U_{INH}) then the latter cannot be estimated within the total uncertainty (U_T). Therefore, the selection and optimisation of the analytical method and the design of the homogeneity test are essential. On the other hand, inhomogeneity becomes negligible as in many certifications the interlaboratory study leads to large uncertainties including a possible inhomogeneity as several samples are used.

In case of spot contamination of one element or substance in a solid matrix, the extrapolation to a tracer is not possible. If spot contamination is suspected e.g. by the origin and type of material or the possible contamination, the analyst can follow the suspect

element or compound in parallel with others. He can try to establish whether there are explainable differences between the respective determinations of U_T . When starting with a very low sample size, regularly increasing the intake but starting in a domain where reproducibility of the method for several elements or substances will remain constant ($U_M = \text{constant}$), if U_T decreases for one element or substance and not the others then spot contamination or the presence of nuggets (small particles with high analyte content) is confirmed. The minimum recommended sample intake will be different for such substances or the highest intake is chosen for all of them.

4.3.1.3. Extrapolations

As demonstrated above, in some situations the minimum sample size cannot be established on the reference material itself. Therefore, it is important that the producer has a good knowledge of the behaviour of his material or has studied it in a preliminary feasibility study. Knowledge in material sciences, biology, mineralogy etc. can be of great help. Several authors have demonstrated that for solid materials a good relation exists between particle size and homogeneity [29–31]. They have shown that with particle sizes of less than 125 μm in a solid material like sediments or soils the homogeneity can be expected down to less than 100 milligrams. In addition when the particle size distribution is also narrow, e.g. in the range of some μm , then homogeneity remains preserved even for long storage periods or can be easily re-obtained by simply shaking the vial for few minutes. The producer has just to assure that the analytes of interest in the material are not subject to migrations (e.g. volatile compounds which would concentrate at the top of a bottle) nor are subject to local degradation (interface material/air where microbes may develop). It must be noted that instability (microbial effects in particular) may generate inhomogeneity within or between vials of (C)RMs.

4.3.2. Within-vial homogeneity study

4.3.2.1. Objectives and limits

A reference material is produced so that each unit allows repeated measurements. It is necessary to verify that all test portions in the unit of (C)RM are identical at least for the parameter to be measured. The within-vial inhomogeneity is the basic investigation on the validity of the homogenisation procedure. It will also have the objective to determine, if possible, the minimum sample intake. As already discussed above the minimum sample size often corresponds to the limit of determination of the method rather than the real inhomogeneity level of the material. The determination of the minimum sample size and a first view on the homogeneity can be obtained already on the bulk material before packaging. The final assessment must be performed on the packed material.

To estimate the within-vial inhomogeneity several measurements of test samples of the same size are performed. Up to ten determinations should be done in the most repeatable manner. The coefficient of variation (CV_{EX}) or the 95% confidence interval

(CI_{EX}) between results will give an estimate of the inhomogeneity (CV_{WITH} or CI_{WITH}) if the uncertainty of the method (CV_M or CI_M) is known:

$$CV_{EX}^2 = CV_M^2 + CV_{WITH}^2$$

$$CI_{EX}^2 = CI_M^2 + CI_{WITH}^2$$

If the method contribution to the total experimental CV (CV_{EX}) or CI is known e.g. nondestructive testing, then the inhomogeneity of the material for the tested sample size can be determined. If inhomogeneity can be demonstrated for a given sample size, several conclusions can be drawn:

- the material is thrown away as it does not achieve the required homogeneity performance in view of its final use;
- the material is processed again to improve the homogeneity;
- the material cannot be improved, the homogeneity is tested on a higher sample intake;
- the inhomogeneity is taken into account when assessing or certifying a property value in the material.

The first strategy is radical and costly but may be necessary when the homogeneity is a major characteristic of the material. Such situations are encountered in material testing, surface measurements, e.g. surface homogeneity of alloys for SIMS where the inhomogeneity is a property of the material itself.

Reprocessing is the most logical strategy but may be hampered by the absence of a better performing, suitable homogenisation technique with the necessary representativeness of the material. In most cases the sample intake must be increased so that no inhomogeneity affects the analytical result. The lowest sample intake for which no inhomogeneity can be detected anymore will be a mandatory factor for the use of the CRM. This approach is also adapted to materials where particles have to remain over a certain size e.g. leaching of soil, or where too small particle size attributes physico-chemical effects (surface activity, static electricity etc.). The latter case where the uncertainty of the assessed property (certified) value of the material is corrected for the inhomogeneity must remain an exception. It happens for surface analysis and rare situations where better materials cannot be produced. Unfortunately, in many cases measurements are destructive, and it is difficult to properly assess the method uncertainty. In these cases the experimental CV or CI and the method CV or CI do not allow one to estimate properly the within-vial inhomogeneity. Consequently, it remains an individual and case to case judgement of the analyst whether homogeneity can be admitted or inhomogeneity suspected. For solids, experience gained on similar materials can give a good answer. In no way however should the certified value be associated to an uncertainty that is not compatible with the experimental uncertainty of the homogeneity test.

4.3.2.2. Testing for the within-vial homogeneity

All tests must be performed in the most repeatable way: one operator using one instrument in a short period of time for the test. If possible experiments should be done in parallel: all extractions in parallel, if possible one calibration. The content of the

substance quantified must lie largely over the determination limit (this may be a compromise with the size of sample intake).

- Establish the test method uncertainty on n experiments in the most repeatable way (see 4.3.1.1);
- Take from one vial n sub-samples and measure them with the tested method;
- Repeat the exercise on a second vial;
- Compare the results of the obtained CV or CI as discussed above.

If the CVs or CIs are similar there is no reason to suspect inhomogeneity. In this case, the within-vial homogeneity can be repeated on decreasing sample intakes. When very sensitive methods are available (e.g. INAA, SS-ZAAS) the homogeneity can be measured down to some micrograms of material [36,40]. Ingamell [42] has established that the uncertainty due to material inhomogeneity is inversely proportional to the square root of the mass of the sample intake. The Ingamell's sampling constant, based on the fact that the between-sample standard deviations (s) decrease as the sample size increases, gives the optimal testing portion size:

$$MR^2 = k_s$$

Where M represents the mass of the test sample, R the relative standard deviation and k_s the sampling constant corresponding to the mass of sample required to limit the sampling uncertainty to 1% with 68% confidence. The magnitude of k_s may be determined by estimating R from a series of measurements of samples of a mass M . Once k_s is determined for a given sample, the minimum mass M required for a certain standard deviation can be calculated.

The minimum sample intake for which homogeneity becomes a significant uncertainty factor of the measurement is (often) however debatable. The CRM is intended to validate methods used for routine measurements. In daily practice of environmental monitoring, the prerequisite is to sub-sample representative samples (see Chapter 2). The problem lies more in having the possibility of handling large samples rather than extremely low sample sizes. It is another debate when forensic analyses are performed as methods may have to be validated on extremely small samples.

Often the producer of a CRM is confronted with the situation where no real conclusion can be drawn. This is usually the case for trace organic determinations:

- the measurements for the homogeneity study could not be done in the most repeatable way (usually it took more than a day and required more than one calibration);
- there is a small difference between measurement uncertainty determined on the extract and the various sub-samples of material;
- the part of the uncertainty due to the extraction or digestion (U_E in the equation above) cannot be determined;
- between various substances or isomers having similar concentrations the method CV (or CI) is sometimes higher or lower than the within-vial homogeneity CV;
- in both vials studied the CV may be different for the same substance and from one substance to the other;
- increasing the sample intake does not change the results significantly.

In such cases the analyst is in fact confronted with the limit of the approach. The measurement tool he uses (which might be the best one the state of the art allows) is not able to deliver an answer on homogeneity of this material.

4.3.3. Between-vial homogeneity

4.3.3.1. Principle and objective

When processed, a material can be subject to drifts in the production. It can happen during the production of a material e.g. a bar of metal alloy changes composition, or a segregation occurs, e.g. denser particles sediment during filling of bottles. The bulk material can be homogeneous, within one vial no difference may be noticed but between vials differences can exist. Such situations should never happen.

For materials destroyed during analysis, it is not possible to measure all individual samples. If the trend in preparation is continuous and known, an extrapolation on the uncertainty of the certified value can be attempted; but this would also decrease the analytical value of the CRM. In the majority of situations drifts or trends are not foreseen and known. Therefore, it will be necessary to measure samples all over the packaging procedure. The between-vial homogeneity study has the objective to verify that no difference exists in the parameter of interest (concentration of the element or substance) between sets. It may also verify that the matrix composition remains similar. The objective of the within-vial study was to verify the homogenisation procedure and estimate the minimum sample intake if necessary. The between-vial homogeneity assesses that the material is suitable for comparability between laboratories.

If a demonstrated inhomogeneity exists between samples this must be reflected in the uncertainty attributed to the assessed or certified value.

4.3.3.2. Testing for between-vial homogeneity

As mentioned on the chapter on homogenisation, for each batch or set of material processed (e.g. one bottle on a batch of 50 bottles filled between two mixing steps), one is set aside. This approach is to be preferred to random sampling of vials. In the latter case it will not be possible to detect trends in the procedure.

To estimate the between-vial inhomogeneity, measurements of one test sample per vial of the same size are performed. The determinations must be done in the most repeatable manner. If samples were produced over several batches, as many measurements must be made as batches handled; sometimes several days will be necessary. The coefficient of variation (CV_{EX}) or the 95% confidence interval (CI_{EX}) between results will give an estimate of the inhomogeneity (CV_{BTW} or CI_{BTW}) if the uncertainty of the method (CV_M or CI_M) is known:

$$CV_{EX}^2 = CV_M^2 + CV_{BTW}^2$$

$$CI_{EX}^2 = CI_M^2 + CI_{BTW}^2$$

If the method contribution to the total experimental CV_{EX} or CI_{EX} is known, e.g. nondestructive testing, the between-vial homogeneity of the material for the tested sample size can be determined. If inhomogeneity can be demonstrated for a given sample size, several conclusions can be drawn:

- the material is thrown away as it does not achieve the required homogeneity performance in view of its final use;
- the material is processed again to improve the between-vial homogeneity;
- the procedure cannot be improved and inhomogeneity is taken into account when assessing or certifying a property value in the material.

Again, the first way is radical and costly but may be necessary if reprocessing is not feasible. When the inhomogeneity affects the intrinsic value of the material too much it must be reprocessed. Such materials may be needed in method validation for environmental monitoring where natural solid materials (e.g. plants, soils, lichens) with very complex structures are analysed. If highly precise measurements of ultra-trace contamination over large geographic areas are performed, very accurate between-laboratory comparability is needed to assess trends in contamination or decontamination. Reprocessing is the most logical strategy but may be hampered by the absence of a better performing mixing and homogenisation technique. The latter case, where the uncertainty of the assessed property (certified) value of the material is corrected for the inhomogeneity, must remain an exception. Systematic inclusion of an uncertainty factor, due to between-vial inhomogeneity, into the certified uncertainty is a way of hiding inappropriate production processes.

Unfortunately, as for the within-vial tests because of the destructive aspect of chemical measurements, it is difficult to assess properly the method uncertainty and consequently the inhomogeneity factor. In between-vial testing the number of samples to be measured may be much more important than for the within-vial study. Sometimes several days or weeks may be necessary to fulfil the task. Consequently, the method variability may be more important and cannot be compared directly to the method uncertainty established above. In such a situation, the between-vial variability should be compared to the within-vial homogeneity results. It is sometimes even difficult to compare the results of the within-vial study with the data of the between-vial test e.g. because the duration of both studies were very different. Here too, it remains an individual and case to case judgement of the analyst whether homogeneity can be admitted or inhomogeneity suspected. Again, experience gained on similar materials can bring a certain answer. In no way should the certified value be associated to an uncertainty that is not compatible with the experimental uncertainty of the homogeneity test.

4.3.3.3. *Practical implementation*

All tests must be performed in the most repeatable way. Again experiments should be done in parallel: all extractions in parallel, if possible one calibration. If necessary and possible (stability problems), all extracts produced in parallel should be stored in the dark at low temperatures. Purification and final determination should be done also in parallel. It is preferable to study the between-vial homogeneity with the same sample intake as for the within-vial test. This should allow more direct comparisons of results.

- select the n vials during the filling procedure on the totality of N samples prepared;
- take from each of the n vials one sub-sample and measure all sub-samples in the shortest possible period of time;
- establish the method uncertainty on n experiments;
- calculate the mean μ and standard deviation s of your n measurements;
- compare the results of the obtained CV or CI as discussed above.

Conclusions to be drawn

If the coefficients of variation are similar there is no reason to suspect inhomogeneity. In this case, the between-vial homogeneity is acceptable. Often the producer of the CRM is confronted with the situation where no real conclusion can be drawn. This is usually the case for trace organic or organo-metallic determinations:

- there is a small difference between the measurement uncertainty determined on the extracts or digests, the within-vial and the between-vial test;
- various substances or isomers having similar concentrations show a method CV (or CI) sometimes higher, sometimes lower than the corresponding within-vial CV;

In the first case, if the uncertainty of the assessed, i.e. certified, value is similar or larger than the between bottle homogeneity, there is no reason to include an inhomogeneity factor into the certified value. Similarly, there is no reason to suspect (unless scientific arguments exist) that one organic substance behaves differently from the others. Therefore no correction has to be applied.

Sometimes a trend is detected between the vials; a systematic increase or decrease in the content of an element or substance appears between vials. Two explanations are possible. Either the production of the material has suffered from a drift or the measurement method has itself drifted. To differentiate between both reasons, the analyst must perform the measurements between samples in a non chronological manner. As samples should be taken regularly during the packaging procedure and are labelled it is possible to arrange their analysis in a randomised way. If a random selection is performed for the between bottles test the drift in production may be overlooked. Figure 4.8 illustrates the differences in conclusion in case of production and method drift. It indicates that in addition to the dispersion of the results, the analyst must also identify the evolution of the results in terms of absolute quantity.

Effect of between-vial inhomogeneity on certified values

The between-vial homogeneity study has to guarantee that samples available to all users are identical. If there is a small quantified difference between the samples this must be taken into account in the uncertainty of the certified or assessed value. As already mentioned, the situation is rarely as simple. Very inhomogeneous materials never arrive at the stage of certification (at least in BCR) as production rules and pre-knowledge avoids it. Very homogeneous materials where inhomogeneity cannot be established and the corresponding minimum sample intake cannot be determined occur frequently for CRMs certified for trace elements. For the majority of other solid phase CRMs, in particular in the field of trace organic substances, precision of measurement methods does not allow rapid conclusions. In all these cases the method CV_M and the homogeneity test CV_{BTW} are similar. Based on material science knowledge, production

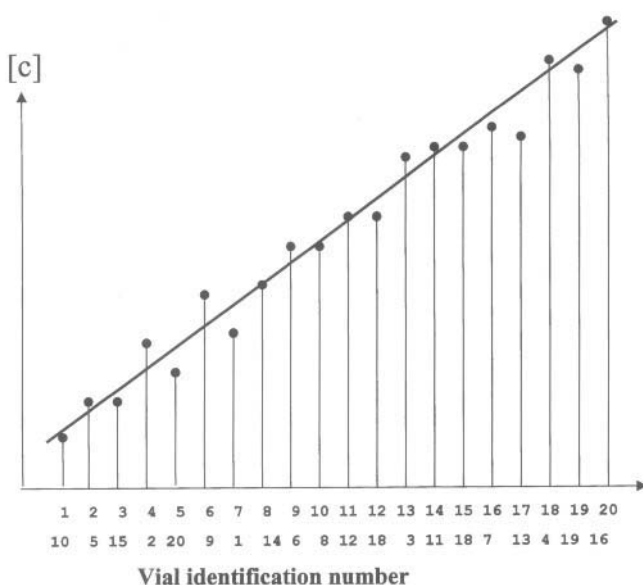


Fig. 4.8. Drift of production method or analytical method?

When verifying the between-vial homogeneity it is preferable to analyse the samples in a random way (lower sequence of vial number). When analysing the samples in the same sequence as they are filled (upper sequence of vial number) there is a risk of confusing a measurement drift (due to the analytical method) and a production trend. It is evident that the samples must be identified during filling and must be set aside regularly during this filling procedure.

skills, experience from other (C)RMs, it is often possible to eliminate any reason for inhomogeneity and no correction of the uncertainty of the certified values is necessary. For powdered solids, which are the most critical environmental (C)RMs, particle size analysis gives an excellent guarantee on the level of homogeneity achieved. Figure 4.9 gives examples of graphs describing the particle size distribution of various CRMs produced by BCR. When large particles are required e.g. for sequential extraction, (C)RM problems arise. It is the role of the certifying body to apply adapted rules for correcting the uncertainty of certified values by inhomogeneity contributions.

The Guide to the Expression of Uncertainty in Measurements (GUM) advises on how such a statistical calculation can be done [43]. Unfortunately, the GUM as well as many ISO-REMCO Guides is not really user friendly. Very few examples appealing to analytical chemists are given. As already stated, inhomogeneity is not easy to measure in powdered environmental samples. Therefore, it could fall into the category of type B uncertainties. The GUM (§ 4.3, page 10) defines such uncertainty inputs as defined by judgement. If it is measurable by repeated experiments it falls into the Type A evaluation. Whatever type it is, the use is similar. If U_{BTW} cannot be measured, it could be estimated as being equal to U_{EX} above (sum of inhomogeneity and method uncertainty).

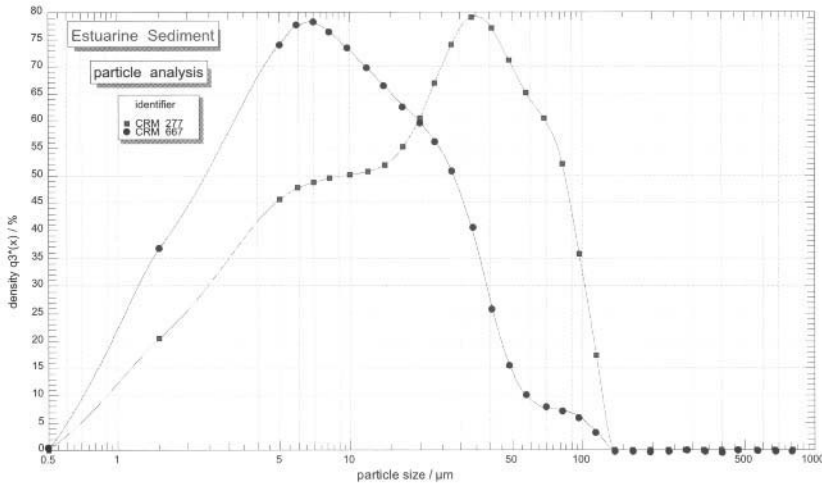


Fig. 4.9a. ■: CRM 277 ball milling, ●: CRM 667 jet-mill

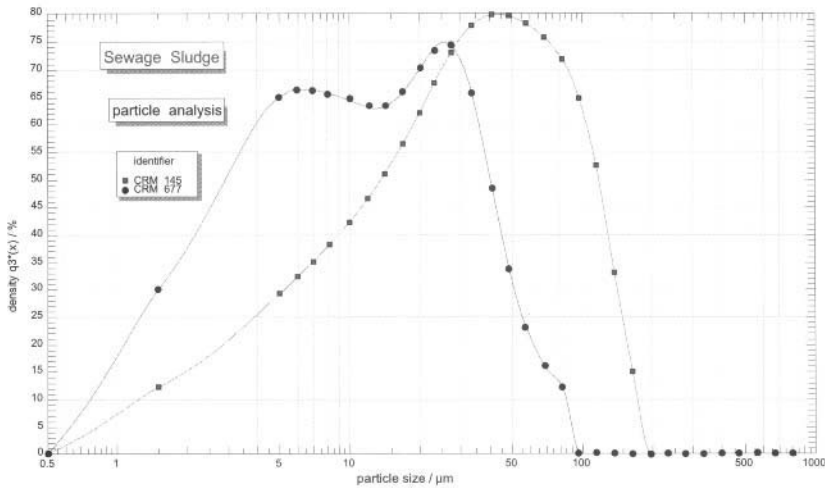


Fig. 4.9b. ■: CRM 145 ball milling, ●: CRM 677 jet-mill

The total uncertainty of the assessed or certified value could be calculated as a combined uncertainty as follows (GUM §5: determining combined standard uncertainty, pp. 17–20 and §6: determining expanded uncertainty, pp. 21–22):

$$U_{\text{CERT}} = k \cdot (U_{\text{BTW}}^2 + U_{\text{MEAS}}^2)^{1/2}$$

where U_{CERT} is the uncertainty of the certified value, U_{BTW} the measured inhomogeneity (type A) or the uncertainty U_{EX} if U_{BTW} is not really measurable and U_{MEAS} the standard uncertainty calculated from the certification measurements. The factor k is an expansion factor which should be set equal to 1 when the certification is

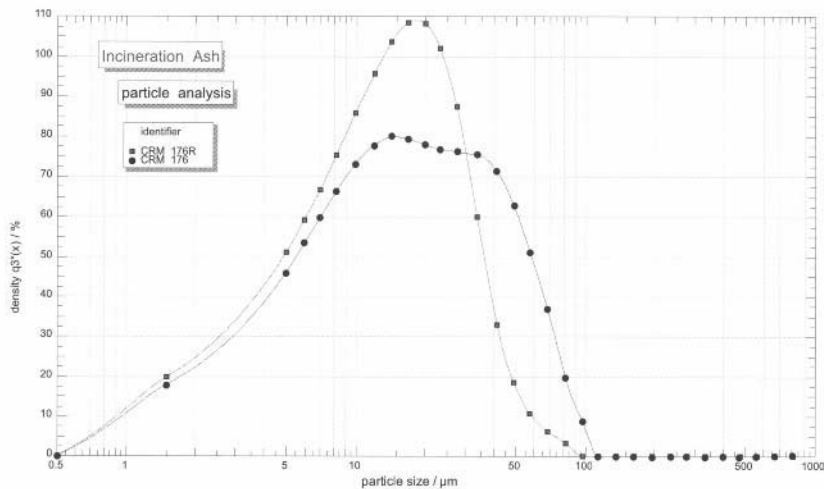


Fig. 4.9c. ●: CRM 176 ball milling, ■: CRM 176R jet-mill

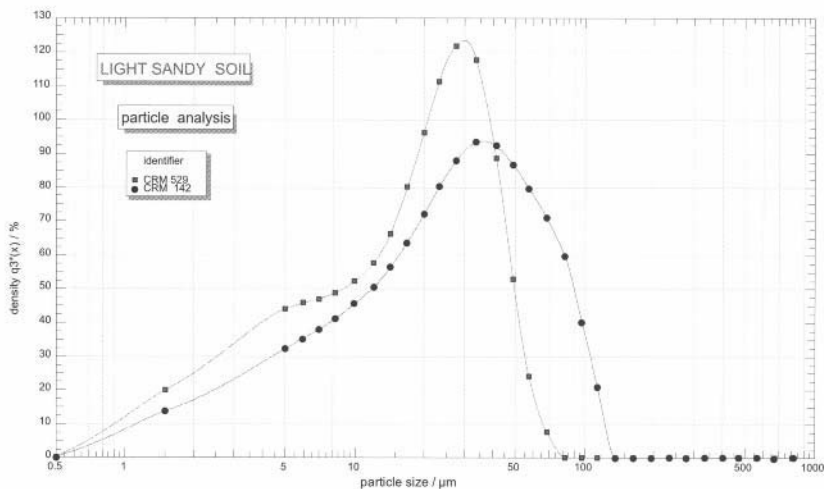


Fig. 4.9d. ●: CRM 142 ball milling, ■: CRM 529 jet-mill

based on an interlaboratory study, unless serious reasons (e.g. commercial, health, or safety security factor, requirement in a standard, etc) justify it.

ISO Guide 35 uses another approach, which is similar but introduces a k' factor in the uncertainty of the homogeneity U_{BTW} [44]:

$$U_{BTW} = k' \cdot s$$

where s is the standard deviation of the n between homogeneity measurements as stated above. k' is a factor for two-sided tolerance limits, with a proportion p of the population of samples to be covered (usually 95%) and the probability level $1-\alpha$ of the

test (usually also 95% rarely 99%). In both cases, ISO 35 and GUM, it is assumed that no other uncertainty components exist which may affect the total uncertainty. If so, they should also be added.

Other ways of expressing the uncertainty exist [43] as recalled by the GUM (§6, page 22) e.g. replacing the above k' by a factor of 2 or 3, lead to the famous two or three s tolerance limits. A factor of 2 is in fact equivalent to k' when the number of measurements n becomes high and corresponds to an approx. 95% level of confidence. A factor of 3 (approx. 99% level of confidence) is very cautious and may increase arbitrarily the final certified uncertainty of the material thus harming its interest for accuracy testing.

4.3.4. Between-vial check through interlaboratory study

Many certifications of reference materials are performed through interlaboratory studies. As discussed in section 5, such studies are planned for the certification purpose under a strict protocol. Such studies include usually from 10 to 20 or more laboratories working independently. Each laboratory receives normally two vials of material and does 3 independent measurements on each vial. This means that often up to 40 vials of the (C)RM are used to establish the certified value. As such it appears that as many samples are tested in an interlaboratory homogeneity study. ISO-Guide 35 recognises that such a study can be used to confirm the between-vial homogeneity of the (C)RM. It recommends the use of a two-stage nested design to test the homogeneity (see Figures 4.10 a and b). Without going into details of the statistical computation of data, it must be stressed that this approach is very practical but has two limitations: a practical analytical one and an economic one.

Analytical limit

If the study is performed by widely differing methods, the variance of these methods may be completely different. In fact, the certification through interlaboratory studies has as its prime objective the use of such different methods to reduce or eliminate all

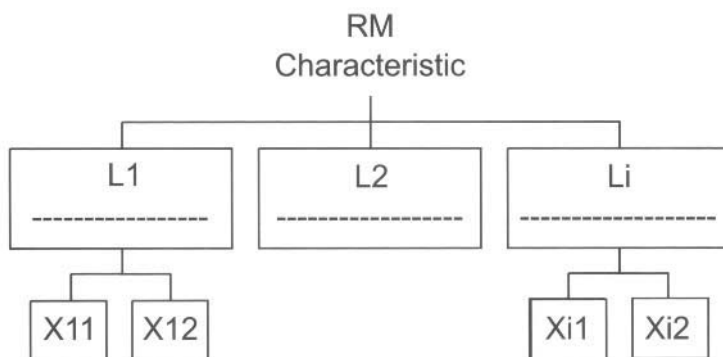


Fig. 4.10a. One stage nested design. Each laboratory receives only one bottle of material and performs several measurements on them (2 in this case). This design does not allow one to test the between-bottle homogeneity within one laboratory and reduces the between-laboratory input.

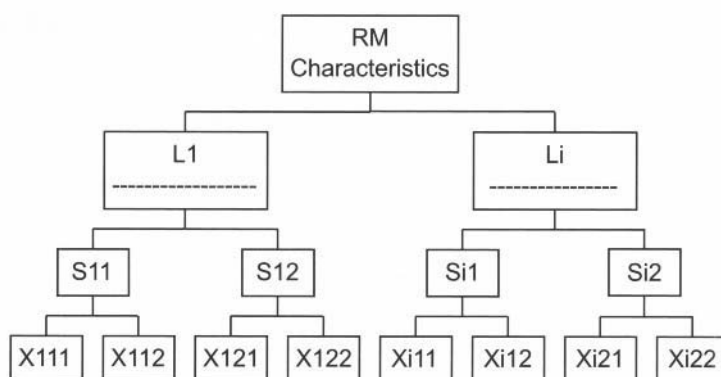


Fig. 4.10b. Two stage nested design of an interlaboratory study allowing the verification of the homogeneity of the RM. (L: laboratory number 1 to i, S: sample bottle number 1 or 2 of laboratory i, X: test sample of bottle 1 or 2 of laboratory i). In total $2i$ bottles are used, and in total $4i$ measurements are performed. If the tested bottles are selected regularly during the filling procedure, a good estimate of the between-bottle homogeneity can be given.

sources of bias linked to one particular method. As can be seen from Figure 4.11, variances may differ enormously e.g. between IDMS, DPASV and AAS for Pb in mussel tissue. Information on between-vial homogeneity is therefore reduced. In addition, certification measurements with such different methods cannot be done always on small sample sizes e.g. FAAS needs larger samples than GFAAS or INAA because it is often less sensitive.

Economic limit

The interlaboratory homogeneity test performed in parallel to the certification has also an economic drawback. If after the study the material is finally considered as very heterogeneous, i.e. homogeneity is an important component of the total uncertainty of the certified value, then the material may be useless. In that case all the money spent in the certification is lost. It is the policy of BCR and many other major producers to withdraw inhomogeneous materials from the study before certification starts. This can only be done in preliminary tests. The interlaboratory certification study may only confirm that no inhomogeneity affects the material (within the above restrictions) if the homogeneity study could not reach a clear answer (e.g. the method used to test homogeneity was not very precise, or doubts remained because of outlying data). In that case the between-laboratory variance is compared to the between-vial variance.

4.3.5. Conclusions

Testing the homogeneity of a (C)RM is an essential aspect of a reliable production. It will help the material to fulfil its space and time duty to assure comparability within a single laboratory over longer periods of time and between laboratories. It is a task to be performed by the producer who has the duty to:

- indicate to the user the minimum possible sample intake of the CRM

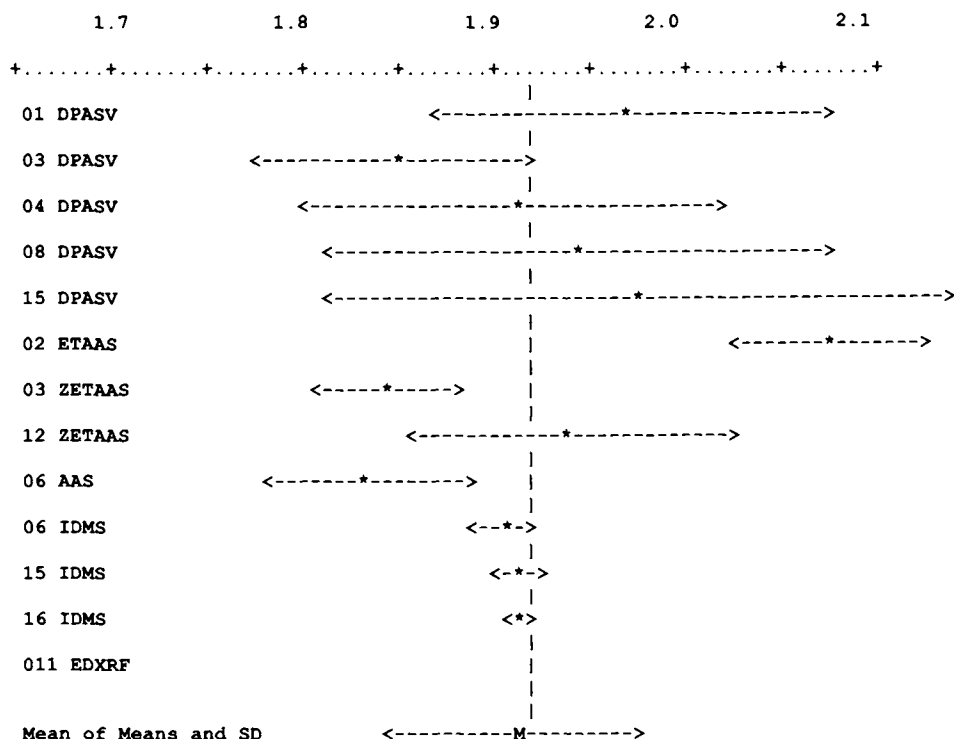


Fig. 4.11. Bar-graph presentation of results obtained in the certification of lead in mussel tissue BCR-CRM 278. The horizontal dotted line signifies the standard deviation of the set of data and the vertical dotted line the certified value (mean of means). The figure shows that the SD of the DPASV and of the AAS methods are much larger than those obtained with IDMS. These differences would influence the evaluation of the homogeneity of the material through a two-stage nested design as proposed by ISO Guide 35.

- the possible source of uncertainty linked to non comparability between samples taken from various vials.

As it can be seen from the theories of estimating the uncertainty component due to inhomogeneity, first of all sound analytical arguments should always precede and prevail over statistics. Inhomogeneity should not remain a simple fact but its existence should possibly be explained by the producer. In all cases, homogeneity studies must be performed. If any correction has to be applied because of the (in)homogeneity the way of calculating it and its value should be clearly stated. If possible all raw data used should be available so that a user may estimate the correction applied. As can be easily imagined, serious certifying laboratories always use the best available measurement methods for homogeneity testing. Differences between uncertainties in homogeneity studies and certification exercises always remain small. In several years of certification of solid materials for environmental monitoring in BCR the uncertainty of a certified parameter in a material never had to be adjusted for significant figures. This is also due to the fact that really inhomogeneous materials were never allowed to reach the stage

where certification was discussed. Poorly processed materials were always redone or discarded. This follows the recommendations of ISO-REMCO. Inhomogeneous materials should not simply be certified with expanded uncertainties without a severe chemical and technological scrutiny and sound arguments.

Statistics should not serve to hide bad technical and analytical work.

4.4. STUDY OF THE STABILITY

The composition of the material and the parameters investigated should remain unchanged over the entire period of use of the material. The study of the material's stability in time will mainly depend on its role. If the material is foreseen to be used in a short term interlaboratory study the stability has to be monitored only over the real duration of the exercise and additionally mimic situations which may be encountered during its short lifetime e.g. transport under severe climatic conditions. Stability studies may vary from some days to several years. The sources and risk of (in)stability should be studied or known before the RM is produced. They should be monitored on the batch of (C)RMs. This study can only be performed by scientists aware of the type of material and its potential sources of instability. Consequently, collaboration with the end-users is mandatory.

4.4.1. Objectives

As stated in the section on stabilisation, sources of instability can be of various origins. Knowing these sources allows one to put the material into a situation favouring the start of instability and follow the evolution. In parallel, it is important to have available a situation where no instability can occur. The latter serves as a reference situation for the sample compared to the destabilised material. If the (C)RM has been properly prepared and is packaged in an adequate vial the sources of instability are reduced. Studies based on the effect of light and humidity can be avoided as these factors can be smoothly eliminated. When prepared, protection from light and humidity can be easily realised by using simple storage vials. Maintaining acceptable humidity and light exposure is also easily realised in a simply equipped laboratory. Protection from effects of increased temperature is difficult, in particular during transport. Increased temperatures may favour chemical reactions and microbial growth. The producer has the duty to establish the behaviour of its material in time and under some destabilising conditions. These studies must be performed on the candidate CRM and on the parameter to be certified. Extrapolations are possible but their validity must be assessed. The study of the stability or instability can be performed following two basic principles:

- monitor the disappearance of the element or substance in the samples;
- monitor the appearance of known degradation products.

In both situations the stability or instability is verified by an analytical method. As for the verification of the homogeneity, the reproducibility of this method is of crucial importance. It makes no sense to be concerned about degradations of some few percent of an analyte if the method does not allow one to detect differences of less than 10%!

Therefore, for some well-known matrices and some perfectly stable substances or elements, common sense is often better than any measurement. Also linked to the precision of the analytical method, it is always better to look for the appearance of degradation products with a method with optimised sensitivity rather than checking for the disappearance of few percent of a highly concentrated substance. Unfortunately, degradation products are not always known or they may already exist naturally in the sample at high concentration levels. Nevertheless, it is sometimes possible to find cases where degradation products are of help e.g. degradation of p,p'-DDT into p,p'-DDE in BCR-CRM 115 [12] and in BCR-CRM 598 [14] or arsenobetaine into dimethyl arsinic acid in BCR-CRM 627 [1,2].

4.4.2. Multiple temperature approach

As stated above an increase in temperature represents one source of potential instability. Therefore, it is interesting to realise a challenge test on the material to see how it behaves over longer storage periods at increased temperatures. By comparing samples stored at various temperatures, instability due to this factor can be produced and measured. The BCR is currently performing studies of the stability of CRMs at room temperature and at +35 or 40°C up to two years, sometimes more. If the material (e.g. foodstuff) is known to be a good substrate for the growth of bacteria or moulds, a temperature of +35°C may be preferred to +40°C. In order to avoid drifts in the analytical methods used in these long term studies, a particular precaution has to be taken. To compensate for method drift, samples stored at -20°C or less (at this temperature chemical or biological changes are negligible) are also measured and serve as reference. The results obtained at +20°C may lead to an assessment of the sample stability at ambient laboratory temperature. The results obtained at +40°C are used to assess the worst case conditions (e.g. during transport). It is indeed assumed that a sample stable at +40°C during one year may be stable at +20°C for a much longer period even if this cannot be mathematically demonstrated. Stability tests may be carried out at the beginning of the storage period and after various time intervals e.g. 1, 3, 6 and 12 months or more. At these predetermined time intervals they are analysed for the substances to be certified. As already mentioned, samples stored at -20°C (or less) are used as reference for the samples stored at +40°C and at +20°C respectively. This is done by expressing the results as the ratios (R_T) of the mean values (X_T) of measurements made at +20°C and +40°C, respectively, and the mean value ($X_{-20°C}$), from determinations made at the same occasion of analysis on samples stored at a temperature of -20°C are calculated. The overall uncertainty U_T is obtained from the coefficient of variation (CV) of the 3 to 5 measurements obtained at each temperature:

$$R_T = X_T / X_{-20°C}$$

$$U_T = (CV_T^2 + CV_{-20°C}^2)^{1/2} \cdot R_T$$

In the case of ideal stability, the ratios R_T should be 1. In practice however there are some random variations due to the error on the measurement. If the value 1 is contained between $R_T - U_T$ and $R_T + U_T$, one can assume that the samples are stable under these

storage conditions. This approach developed by Griepink et al. [45] has the following advantages:

- it definitively recognised the (in)stability as source of uncertainty (problem) in CRM production;
- it developed a way, simple but easy to apply, to eliminate long term uncertainty of methods by introducing the measurement of a reference sample stored at a temperature where no instability can be expected and to express results in terms of ratio;
- it associated a combined uncertainty to this ratio.

The method was developed for materials in the field of organic chemistry and microbiology where instability was likely. Unfortunately, in both fields, analytical methods show poor precision, so that the power of the method was limited. How does one detect a 1% decay over a year with a method having a repeatability of 10% or more? Considering these limitations, the analytical chemist has no choice other than discarding any RM showing the slightest instability at a given temperature. An alternative consists in storing and transporting it at a lower temperature where this instability does not exist. Such materials have been identified over the years by BCR and have been treated accordingly [46,47]. Lack of precise measurement tools have sometimes prevented the estimation of further possible decays, which could appear over longer storage periods or which produce decreases or increases that are hidden by the method uncertainty [48].

4.4.3. Analytical methods used for the study of stability

Long-term reproducibility is the prime quality of an analytical method used for the study of stability. Stability must be performed on the element or substance to be certified. Extrapolations on the stability of tracers are of little interest. If the uncertainty of the method for an analyte is poor, even for large sample intakes, the analyst must refer to the experience on similar materials with higher contents or to the general chemical properties of the substance or element. In any other situations certification may be impossible. Such difficulties are mainly encountered in organic or organo-metallic analysis. Experience has shown that some substances may be stable in a matrix but unstable in another even similar one [46–47]. All analytical methods suffer from long term reproducibility. In some cases analysts have developed tools and tricks to minimise this effect.

For inorganic determinations INAA is often a good choice for studying stability. This technique allows one to avoid a sample pretreatment step for some elements and matrices and consequently excludes the uncertainty due to this step. In addition, a fine quality control can be achieved by performing counting statistics [36]. Long term precision of less than 1% can be achieved for many trace or ultra-trace elements, giving INAA a leading position for precise studies. Therefore, INAA has often been applied for the study of the stability of certified reference materials. IDMS could also be used for this purpose as it is a relative method (isotope ratios) but it is hardly economically sustainable for such studies.

For organic determinations, the same difficulty exists for the stability studies as for the homogeneity studies, with one major drawback. The instability is much more likely

to occur for organic substances than trace elements. Therefore, stability studies for organic substances are of primary importance. Analytical methods, specially optimised for their reproducibility, rarely exhibit precision better than 5%.

The reference to samples stored at a low temperature has its limitations. This technique is not valid any more for water or any solutions in which certain compounds may precipitate and may not re-dissolve reproducibly upon warming up. In such circumstances other adapted long term reproducibility measures and reference samples have to be developed e.g. detection of the appearance of products of chemical reaction or metabolites. These various considerations demonstrate that stability of (C)RMs cannot rely simply on measurements but should also rely on common sense and material sciences. Preliminary behaviour studies of materials, matrices as well as substances to be certified must be performed. Finally, it is necessary to involve in such studies all those who have experience on the material and its behaviour. Ask a milk specialist about the behaviour of milk, not a metrologist or a statistician. The limitation on precision of methods also shows the difficulty of predicting the behaviour of a material on analytical grounds. Therefore, it is highly doubtful to ask that such methods establish any law on the behaviour of a material and to calculate or correct a certified value based on the 'measured decay' of the material. Consequently, it is very difficult if not foolish to state expiry dates on the basis of results of such methods. Other means have to be used. Stability studies should remain challenge tests, performed to verify that a material has no demonstrable instability, at least at some given storage conditions. If instability is likely, the material should be discarded or studied under other conditions.

Besides the preliminary stability study, regular checks should be carried out over the entire lifetime of the material. In some rare situations, over the certification projects conducted under the BCR activities, examples of 'instability' of substances was demonstrated at certain storage temperatures. Figures 4.12a-b on organochlorine pesticides in animal feed (confirmed in fish oil) and 4.13a-b for polycyclic aromatic hydrocarbons illustrate such examples. For p,p'-TDE the increase in content is generated by the decomposition of p,p'-DDT. A similar effect is noticed for o,p'-DDT which fully disappears over a period of six months at +40°C [12,14]. For benzo(a)pyrene it could not be demonstrated whether the decrease in content noticed was due to a decomposition of the PAH or to a decrease in its extractability due to the change of the physical stage in the matrix [46,47]. These materials looked stable at lower temperatures; usually already at +20°C. Consequently they are stored at temperatures of +4°C or less.

4.4.4. Isochronous studies

In the stability study described above, the samples are stored for long periods at various temperatures. After a given period of time e.g. 1, 3, 6, 12, 24 months etc., samples stored at e.g. +20 or +40°C are taken out of their storage cupboard and analysed in parallel with those stored at -20°C. An alternative method consists in storing all samples at -20°C (or less) and placing them for a given period of time at the higher temperatures e.g. 0, 1, 3, 6, 12, 24 months before the date of analysis. It consists, as shown in Figure 4.14, in the same storage time at higher temperatures as in the first approach but allows one to plan all measurements at the end of the study. Measurements are then performed

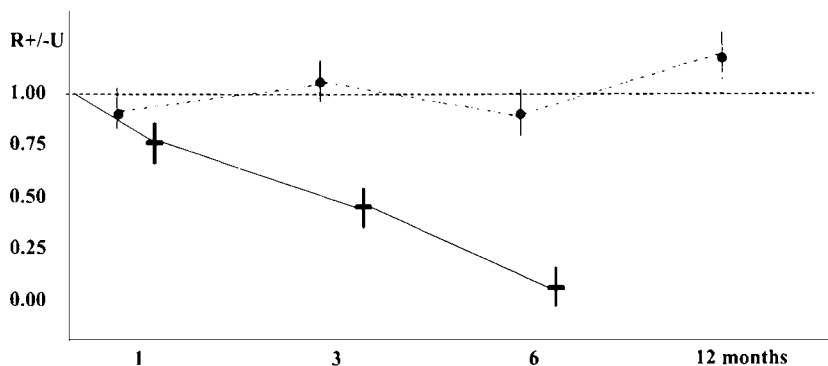


Fig. 4.12a. Effect of temperature on the stability of o,p'-DDT in animal feed BCR-CRM 115. The dotted line (■) represents the ratio and combined uncertainty (vertical line) of each of three measurement results (performed at 1, 3, 6 and 12 months) and obtained at +20 °C versus -20 °C. Ratios of measurements at +37 °C (+) show a rapid decrease of o,p'-DDT. An inverse picture could be found when monitoring o,p'-TDE which is the degradation product of DDT. DDT was considered to be stable at +20 °C. Definitions of R and U are given in the text.

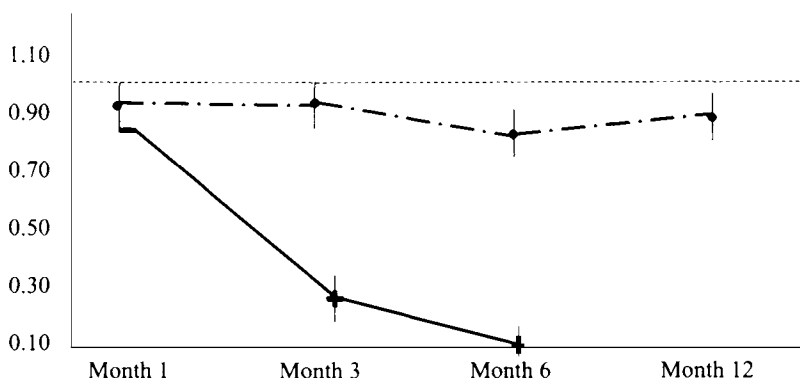


Fig. 4.12b. Effect of temperature on the stability of heptachlor in animal feed BCR-CRM 115. The dotted line (■) represents the ratio and combined uncertainty (vertical line) of each of three measurement results (performed at 1, 3, 6 and 12 months) and obtained at +20 °C versus -20 °C. Ratios of measurements at +37 °C (+) show a rapid decrease of heptachlor. Heptachlor was considered to be stable at +20 °C. Definitions of R and U are given in the text.

in a shorter period of time. Economically, this approach is more suitable because the study can be planned beforehand and the laboratory is concentrated on this task. It does not eliminate all reproducibility problems because up to 50 or more samples must be analysed. For the determination of organic substances this may take several days or weeks. Therefore, the argument for better precision is only marginal. Nevertheless, this approach certainly represents an advantage for managing repeated challenge tests in the laboratories in charge of maintaining large stocks of CRMs. Pauwels et al. have proposed such an approach for the control of the stability of BCR CRMs [49]. For

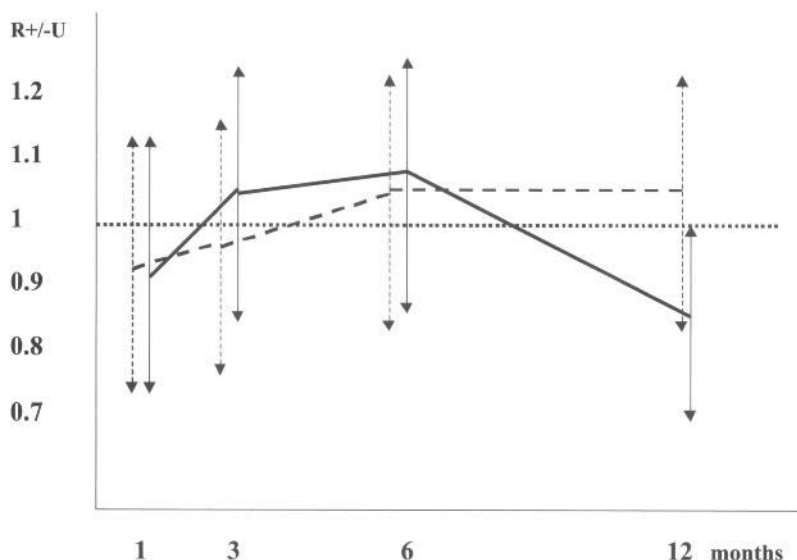


Fig. 4.13a. Stability of anthracene in industrial contaminated soil (BCR-CRM 524) after 1, 3, 6 and 12 months storage at temperatures (T) of +20 and +40 °C. The results are expressed as a ratio (R) of mean values of 5 measurements performed on samples stored at temperature T and 5 samples stored at -20 °C. The combined uncertainty is calculated as explained in the text. A clear decrease can be noted at +40 °C after 12 months. No instability can be noted at +20 °C

CRM suppliers such isochronous tests can be planned and can be repeated over time. For the study of new materials this approach is not suited as the probable instability is only known at the very end. For the development studies on storage and behaviour of the material rapid answers are necessary.

4.4.5. Extrapolations through Arrhenius law

It has always been a strong hope for those in charge of developing, certifying and servicing CRMs to dispose of a kind of mathematical relation which would foresee the limit of stability of the CRMs from a challenge test. A particular equation should determine if the results of the short-term test at elevated temperatures (challenge test) could be used to predict the long-term stability at lower temperatures. The Arrhenius equation has been often proposed for this purpose.

The equation (1), the Arrhenius equation, describes the activation rate k as a function of temperature:

$$k = A \cdot e^{-(E/RT)} \quad (1)$$

where T is the absolute temperature in Kelvin, A a constant, E the activation energy (J mol^{-1}), R the gas constant (8.3143 J K^{-1}). The Arrhenius theory foresees that temperature is an important factor that affects the rate constant of chemical reactions. As shown by Perez Bendito in the FECS curriculum for Analytical chemistry [50], the

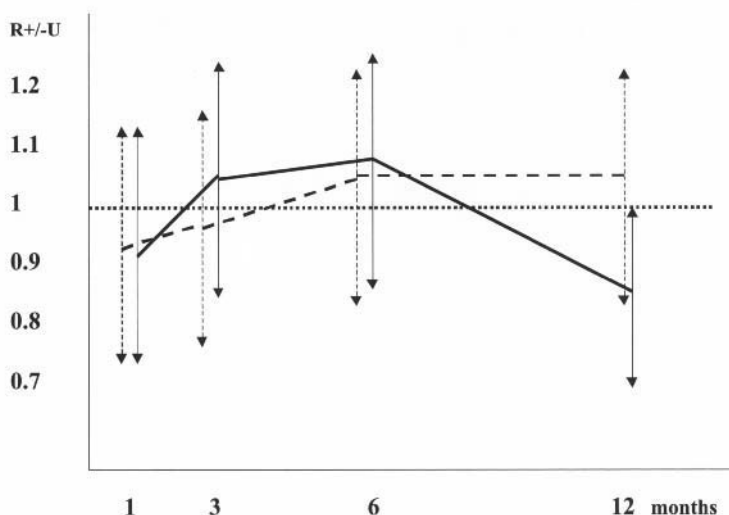


Fig. 4.13b. Stability of fluorene in industrial contaminated soil (BCR-CRM 524) after 1, 3, 6 and 12 months storage at temperatures (T) of +20 and +40 °C. The results are expressed as a ratio (R) of mean values of 5 measurements performed on samples stored at temperature T and 5 samples stored at -20 °C. The combined uncertainty is calculated as explained in the text. A decrease of about 40% can be noticed at +40 °C after 12 months. No instability can be noted at +20 °C

rate constant for homogeneous reactions increases by a factor of 2 to 3 when increasing temperature by 10°C in the vicinity of room temperature. In reality, it is difficult if not impossible to extrapolate such a situation and behaviour to stability problems of molecules in CRM matrices. Nothing indicates that the reaction rate increases in the same manner in such complex systems (materials in a dry stage). It can be supposed that the reaction rate is less affected. It would mean that increasing the temperature by +10°C would not increase the reaction rate by a factor of 2 or 3 but less. This would mean that chemical reactions would affect the materials less. As a consequence, if the stability study does not show any effect of storage at higher temperatures e.g. +40°C over e.g. 1 year, compared to +20°C it could be estimated that more than a factor of 6 (difference of +20°C) of security for stability can be expected. This would mean stability for more than 6 years.

Havelaar and co-workers [16] following studies launched by Pike et al. [51] have studied the applicability of the Arrhenius 'law' for microbe stability in spray-dried milk powder. They have adapted the theory to the particular situation of microbial decay.

$$-b = A \cdot e^{-(k/T)} \quad (2) \quad N(t) = N_0 \cdot e^{-bt} \quad (3)$$

where N is the number of cfp, -b replaces k in equation and represents the rate constant of decrease in viability, t the time. When developing the CRMs for microbiology, one of the major challenges was to stabilise the microbes in a medium and predict the behaviour at long term. The Arrhenius equation was of limited help. Depending on the

Temperature T in °C	Number of bottles	Total number of bottles stored at each temperature				Total number of bottles at the time of measurement
-20	100	80	60	40	20	20
+4	0	+5	+5	+5	+5	20
+20	0	+5	+5	+5	+5	20
+40	0	+5	+5	+5	+5	20
+80	0	+5	+5	+5	+5	20
Total		5	10	15	20	100
	End of production	Duration of storage at temperature T °C				
		12	6	3	1	0
Duration of the study in months		0	6	9	11	12

Fig. 4.14. The road-map for the isochronous measurement of stability shows when the bottles have to be taken out from the initial storage temperature of -20°C to be set at the increased destabilisation temperature. At the end of the production, 100 bottles are set aside at -20°C (could be lower e.g. -80°C). After the homogeneity study (month 0), 5 bottles are stored at each of the studied temperatures. Here a very extensive temperature study is performed ($+4$, $+20$, $+40$, $+80^{\circ}\text{C}$), usually materials are tested at room temperature and $+40^{\circ}\text{C}$ unless feasibility studies have revealed risk of instability. After 6, 9 and 11 months each time 5 more bottles are added at each storage temperature. All bottles (100 in total) are analysed together. Such a study can be planned over three years instead of 12 months. When the analyses do not reveal instability a new study can be started in the same conditions taking as time 0 the end of the measurements of the first study. The disadvantage of such an approach is that the study reveals a possible instability only at the end. Therefore, it is hardly usable for the development and first production of a CRM. It is an advantageous approach for the monitoring of stability by the suppliers as it allows an easy planning of the laboratory work if several materials have to be followed [49].

strains and temperature conditions the correspondence between expected and measured viability of strains was more or less similar. The Arrhenius law was not demonstrated, in the study performed, to be a fully reliable stability prediction test. One of the major reasons might be that microbial viability is a complex problem, not only influenced by temperature parameters. This indicates that the stability study must be based on measurement data but must also be conducted and interpreted in terms of material behaviour, biological arguments and common sense. It is certainly where the input of the experience of chemists and producers is the most crucial.

4.4.6. Expiry dates

Customers frequently ask CRM producers about the duration of the validity of the certified values in a CRM. This is a legitimate question as they need to know and to demonstrate that the CRM they use for their internal quality control is valid. A patient or a physician requires the same from a medicament producer. This legitimate question from customers is a nightmare for CRM producers. As said above, CRMs are produced in large batches. Often the stock is sufficient for several years of supply. This is due to the fact that producing small and large batches involves the same costly feasibility

studies but larger batches allow depreciation of these cost more (or less) easily. Therefore, extrapolation of stability data over long periods is necessary.

The question on the availability of expiry dates is mainly due to the fact that such dates exist for medicaments and food products. If such dates are mainly based on administrative rules, for medicaments scientific knowledge and specific studies are performed on stability. Still several differences exist between CRMs and drugs.

CRMs and medicaments: other role other rules.

CRMs, contrary to medicaments, do not benefit from the existence of adapted delivery systems. In the case of drugs, the industry, wholesalers and pharmacists guarantee, with identified shared responsibilities, the proper delivery of the product. They form an integrated professional chain of production and service with legally defined duties. CRM producers rely on postal and other delivery systems which do not endorse the same level of responsibility as the delivery system of drugs. Therefore, the guarantee of CRM producers can only be given as long as the CRM is in their facilities. Afterwards, they can only select the most appropriate delivery system, give advice on best practice and use of the CRM.

Having said that and presuming that transport and storage at the user's place are done properly, it is difficult to develop a technique (analytical, mathematical or statistical) which gives expiry dates. Again, a wrong approach would consist in comparing the situation of CRMs with those of medicaments. Drugs are synthetic mixtures of natural or artificial substances mixed together with so-called additives which form the medicament. The behaviour of the drug is verified in challenge tests following strict requirements from drug enforcement agencies (FDA, Pharmacopoeia). Based on these tests, the producer issues an expiry date for each batch of drug, usually 3 or 5 years for solid forms except e.g. some antibiotics etc, or less for vaccines and other forms of injectable drugs. The given dates do not reflect a quantified decrease in any drug component which would fall under a limit value but indicate a date after which the producer no longer bears any responsibility on the medicament (activity, concentration, degradation of components, toxicity etc.). It is hazardous (but has been proposed) to extend such a system to predict a decrease of a substance and to predict a change in the certified value. It is even hazardous to try to estimate any influence on the uncertainty of the certified value. We would be far away from metrology or even from science [52]. For CRMs, which are intended to be used as validation of trueness of a method, producers may give a date which lifts their responsibility. In order to avoid legal risk, such dates could be limited to short periods, leaving the user with their material and open questions.

'What does one do if such an expiry date of a CRM is passed even if bought by a laboratory some weeks or a year ago? Does the user then have to replace it?'

The user's demand raises questions to the producer. Two types of problems and answers have to be considered.

Analytical and scientific aspects

Instability affects mainly materials (see above) where chemical or microbial activity may degrade either the substance certified or the matrix. Such situations are mainly

encountered with organic, organo-metallic, biochemical or microbial parameters and natural biological matrices. Unfortunately, it is also for these substances that the methods of determination are less precise. Therefore, analytical monitoring is limited to gross degradations (typically 10% per year or more). If stabilisation is difficult and stable conditions hard to achieve it must simply be concluded that the CRM cannot be produced under acceptable economic conditions. If for a given material and conditions (e.g. high temperature for the test) instability can be induced but not demonstrated, storage and if necessary transport must be performed with a security margin. For example, if the material is stable at +20°C but not at +40°C, storage must be performed at +4°C. As suggested by Pauwels in his study on the quantification of the expected shelf life of CRMs, these studies should be performed up to the moment when selling of the CRMs has started [49,52]. It is then up to the storage and selling department to start a new or continue the running challenge tests. Another safe way seems to be a re-certification of the substances when the challenge tests must be interrupted, to compare the new certified value with the previous one. If both values agree it can be deduced that the material was stable over this prolonged period and additional guarantee exists that it will remain stable again. A new challenge test at several temperatures can be organised again for a new period.

Economic aspects

The expiry date becomes a simple problem when the producer is sure to sell all his material in a period where the stability test can still be performed. An answer could be to reduce the size of the batch so that the market can absorb it within two or three years. This would be a situation similar to pharmaceutical production where frequency of production and size of batch are market driven. Unfortunately, CRM production is a costly business. In particular for many matrix CRMs the end-user population is limited. Having larger batches of CRMs allows a decrease in the cost per unit. Decreasing the batch size will increase the cost of the CRM for the end user. Such cost would become unacceptable for measurements performed in highly specialised fields with limited numbers of laboratories (e.g. toxins, dioxins etc).

4.4.7. Short term studies and conclusion

When instability is detected at an earlier stage of the study the material has to be maintained in adequate conditions. In addition, a short-term stability study has to be performed to monitor the behaviour of the material during transport under possibly increased temperature conditions. Short-term and drastic transport conditions may be reproduced by storing the material under temperatures up to 40–45°C for 10 days. This covers the maximum real time transport duration (normal surface transportation for all major European laboratories customer of BCR). The study is performed as above for the long term stability by comparative measurements.

Stability remains a difficult aspect of the production of CRMs, in particular matrix materials for environmental monitoring. Feasibility studies devoted to the stabilisation of the material must be conducted before any production starts. New materials, such as fresh materials, required by analysts increase the problem of

stability. New ways of production and stabilisation have to be developed and need to be funded.

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Chapter 5

Procedures to certify reference materials

As already discussed in Chapter 4, CRMs can be used to assess the precision and trueness of a method. Precision has already been largely studied and improved by using proper internal validation procedures and can be maintained through appropriate control charts. For all those activities, simple RMs are sufficient. To assess trueness, the analyst has to look for external help. One simple way is to purchase a CRM. This CRM will help him to solve his accuracy problems if two conditions are fulfilled. First, he must choose an adapted CRM — representative of the daily routine samples; secondly, it must be properly certified — the certified value must be a good estimate of the ‘true value’ [1]:

‘the certified value should be an accurate estimate of the true value with a reliable estimate of the uncertainty compatible with the end use requirements’.

The certification of the content of a substance of interest must follow strict, transparent and reliable procedures. The reputation of the certifying body will depend on this procedure and the absence of failures. Several valid procedures for the certification of material properties exist. They can be grouped into two categories:

- absolute parameters e.g. physical property, total content of a substance in a matrix, purity of calibration substances;
- method-dependent parameter: the measurement method defines the certified parameter; the demonstration that the method has been fully and properly followed is the basis of the certified value; such CRMs are encountered in microbiology and for all method-dependent parameters already mentioned before (e.g. CRMs for a particular standardised method, fractions of substances in matrices etc.).

The second category of materials is recent and mainly encountered in biological sciences and related disciplines. They also exist in material science testing. In all cases, the certification consists in assigning a value with associated uncertainty to the amount or property to be measured. As already discussed in Chapter 4, the uncertainty of the material may not only be due to the uncertainty of the certification measurements. Additional sources of uncertainty such as heterogeneity can affect the total certified uncertainty. Materials for which heterogeneity between sets is so large that a single value cannot be assigned to all samples but each single set must be measured individually is rarely encountered in disciplines other than materials testing. In such a case each CRM is certified individually by a nondestructive method. This will not be discussed further. For some more information the reader should refer to ISO Guide 35 [1].

Whatever material and property value is to be certified, the proper, stable and homogeneous material must be available.

5.1. ABSOLUTE AMOUNTS

5.1.1. Pure substances

It is out of the scope of this book to study in detail the certification of purity of substances. Nevertheless, it is important to address the topic as the identity, purity and stoichiometry of substances form the basis for traceability and consequently comparability of chemical measurements. In the discussion below the reader will certainly find material worth contemplating for his own daily work at the level of calibration.

As already discussed in other sections, the basic principle for achieving traceability to the absolute amount of a substance is to have this substance available in the purest possible form. This is expressed as 100% purity; *no mass fraction of impurity detectable*. This is rarely realised. Usually, impurities do remain and the analyst has to detect and measure them and possibly eliminate them. The addition of all the measured mass fractions of impurities is subtracted from 100% to give the purity figure. The added uncertainties of all the measurements of the impurities may serve as a cumulated uncertainty of the purity figure.

The difficulty of the certification of purity starts with the impurities that could not be detected. In this case, the analyst must know which potential impurities can be expected. They can be of two types.

- Impurities present in the raw material(s) from which the ‘pure’ substance is derived e.g. raw metal, ore, organic reactants, natural raw material etc. and remaining after the purification process. This category is the most difficult one as raw materials can be of wide origin and quality.
- Impurities resulting from the preparation procedure. They can be products of chemical reactions, e.g. organic or organo-metallic synthesis, or introduced during the production treatment (e.g. contamination by metals from vessel walls or tools in the production of alloys).

The preparation and synthesis of the product to be purified are tasks requiring the highest chemical skills. Re-crystallisation, distillation, fusion, flotation and so on, are all tools, when repeatedly applied, to achieve basic purity. Preparative liquid chromatography (adsorption mode or ion exchange) achieves the purification processes. During and at the end of the preparation and purification process, analytical methods are applied to verify the degree of purity obtained. When a sufficient purity is achieved, it is possible to start the certification measurements to quantify the purity and its uncertainty. Both categories listed above have to be chased.

5.1.1.1. Inorganic materials

In the production of inorganic materials, pure metals or single elements (A^0) are preferred to oxides, which again are more valuable than salts. The latter may suffer severe problems of stoichiometry as the number of molecules of water of crystallisation obtained is not fully guaranteed or may change over time and with frequency of use. Therefore, for salts (chlorides, nitrates, etc.) it is also important to verify and guarantee the stoichiometry of the final product (IR, UV, etc.). To detect impurities, adequate

methods have to be applied. In inorganic analysis many different techniques exist which give access to the content of impurities in a matrix. In playing with them and their limits it is possible to detect nearly all possible sources of impurities. Difficulties remain with metalloids (O, C, N etc.) in metals. As can be seen from various suppliers, degrees of purity of 99.999% are frequently guaranteed. In order to properly certify the purity, the uncertainty must also be calculated. This may be done on the basis of the detection of impurities. The detected impurities should lead to two possibilities:

- technologically it is impossible to eliminate them; the purity is corrected for their measured mass fraction;
- they can be eliminated by a re-purification and the process is restarted.

In both cases the purity figure is corrected. If the (possible) impurities cannot be detected or only at higher levels of content, an uncertainty due to their undetectable presence remains. In that case the purity figure cannot be corrected, but the uncertainty on this figure changes. This situation also leads to two conclusions:

- the limit of *detection* of the method for the undetectable (but theoretically possibly present) single element or substance is so low compared to the other measurable impurities (e.g. preparation uncertainty, uncertainty of other impurities) that it can be neglected;
- the limit of detection is high and it is possible that the element is present in a significant amount but not measurable; then it adds to the uncertainty of the certified purity.

Example: element A has to be certified as pure element. The measurement of the uncertainty gives the following results:

- (a) N, O, C, limit of detection in the 'pure' element <0.001% (mass fraction);
- (b) 50 elements have a detection limit of less than 0.0001%;
- (c) 5 elements show a limit of 0.001%;
- (d) one element is detected at a level of 0.01% \pm 0.0001% (mass fraction).

The element under (d) will help to correct the purity figure (100.00–0.01 = 99.99)%.

The uncertainty of these 99.99% will depend on the detection limits of the other elements and the uncertainty of the detectable substance. For the sake of caution, the detection limit is taken as the potential uncertainty on the purity.

51 elements at 0.0001% and 5 times 0.001%. Taking all these purity measurements the uncertainty will be:

$$U = k(51 * 0.0001^2 + 5 * 0.001^2)^{1/2} = k * 0.0023\%$$

For k factor equal to 3, $U = 0.0069\%$. The result could be a purity figure of:

$(99.983 \pm 0.007)\%$ or less if a less strict coverage factor k is introduced. This simple calculation considers that no other significant uncertainties exist in the entire procedure, e.g. weighing, volumes etc.

5.1.1.2. Organic and organo-metallic substances

Simple cases such as those encountered in the field of metals and other inorganic substances are rarely encountered for organic substances. In this case the detection problem of impurities is double. First the impurities are more difficult to be identified

and secondly, if they are identified, they are difficult to be quantified as methods are usually less sensitive than inorganic trace element methods.

Organic substances are composed of C and H. Frequently elements such as O, N, S exist in their skeleton. Heteroatoms, e.g. P, halogens, As, Se, metals etc., may also be present. The identity of the substance is mainly given by the formula and isomer composition rather than by the elemental composition. More heteroatoms are present and it is easier to characterise the molecule. In general, the identification of impurities first relies on separation techniques and secondly on the detection system. If the detector is specific (e.g. MS) then more information on the impurities is available. To certify the purity of organic substances the analyst will first establish a proper synthesis procedure that generates as few byproducts as possible. Byproducts should be all identified in theory. Reagents, catalysts for the synthesis and solvents should be of the highest purity and specificity to avoid formation of impurities.

When the product is purified, its identity is established by ^1H NMR, IR, FAB-MS, etc. An elemental analysis associated with a fusion point determination by thermogravimetric methods helps to establish the identity of the substance and its stoichiometry. Then the product is analysed on various GC and HPLC phases with various detectors. Impurities are often present in amounts that do not allow ^1H NMR identification.

When identification is done, the mass fraction of the substance in the material can be studied. Detection by FID and some forms of MS allow reaching a mass proportional response and establishing a quantified link between signal, mass of product and impurity. ECD allows the presence of heteroatoms, e.g. halogens, to be revealed. HPLC may reveal the presence of a substance that is not accessible by GC. Atomic spectrometry, as used for the purity of inorganic substances, can help to determine the amount of metal in an organo-metallic substance and the presence of some metals or metalloids.

The principle of the certification of the purity of the substance consists in establishing the purity grade and the uncertainty on this purity. Elemental analysis, GC-MS and GC-FID help to set the purity figure together with atomic spectrometry in the case of organo-metallics. All measurements should be performed at least in triplicate and if possible in different laboratories. All reagents or solvents used to perform the measurements must be of the highest purity: procedure blanks must be blank. Care must be taken to authorise chromatography to run for a long period (at least 24 h) in order to allow long retention time impurities to be detected. HPLC can also help to correct for non-volatile impurities. Unknown impurities detected by GC-MS or GC-FID can be quantified on a mass response basis and are used to correct the purity figure. Peaks in ECD are not mass proportional and will depend, e.g., on the number of Cl atoms in a molecule. They may be used to detect substances that cannot be detected by FID or MS. Similarly, with HPLC we cannot identify and quantify unknown peaks appearing in UV or fluorescence. These detected impurities cannot be used to correct the purity figure but they will participate in the calculation of the uncertainty of this figure. This uncertainty will be a cumulative sum of the uncertainties of the detection of identified and non-identified FID and MS peaks. Other peaks that cannot be expressed in terms of mass fractions will participate in the expression of the uncertainty. If the uncertainty figure becomes too large because too many peaks remain unidentified and non-quantified, the material cannot be used as a calibrant and definitely cannot be certified! In such

cases the preparation must be changed or the material must be re-crystallised or re-purified with other means (ion exchanger for salts, adsorption or exclusion chromatography for organic substances, etc.). Major difficulties exist for substances that can only be studied in liquid media, e.g. liquid chromatography, because detection systems are less powerful than for gas chromatography. LC or Capillary Zone Electrophoresis detectors (MS) are not yet capable of achieving performances comparable to GC detectors. In addition, only few fundamental studies on the strategy to establish purity figures and to calculate the uncertainty have been published. Examples of purity studies for the certification of primary calibration materials have been published by BCR [2,3].

5.1.2. Mixtures of substances

Mixtures of pure substances are mainly used in the calibration of comparative methods (e.g. XRF) and gas analysis. The mixtures are made from primary pure substances (e.g. metals, gases) which are mixed on a mass basis. Whenever it is possible, the preparation procedure of these mixtures should allow certification on the basis of the gravimetric results. The preparation should not lead to losses or contamination. If this cannot be guaranteed then the certification must be based on measurements and follows the same rules as for matrix materials. In this latter case the uncertainty of the certified value will be much larger.

5.1.3. Calibration solutions

When the purity of a substance is assessed or certified, it can be used to produce a certified solution. The substance has to be dissolved in an adapted solvent. 'Adapted' means that it must be 'ultra pure', it must be able to dissolve the substance at the concentration foreseen but it must also be stable enough to allow long storage and usage periods. Basic chemistry will bring these answers. Additional demands due to transport and safety may influence the choice. Highly corrosive solutions are difficult (expensive) to ship. Organic solvents with very low flash points are even refused by transport companies, in particular for airfreight. Low temperature transport is expensive as is the use of safety packaging.

When dissolving the substance, the analyst must have a properly calibrated balance. Preferably it should be calibrated just before use. In that case the uncertainty due to weighing will be minimal and negligible compared to the purity uncertainty. If several substances are mixed in the solution, contaminants present in one substance may affect the exact mass of another substance (e.g. isomers of compounds such as dioxins, mixtures of metals, etc.). The certified value of the concentration must be corrected for the purity figure and for the presence of cross-contaminants. The uncertainty of the value, possibly as a 95% confidence interval, and the significance of the uncertainty must be given.

5.1.4. Matrix materials

The certification of matrix reference materials must follow the strict rules which are described in ISO Guide 35 [1]: 'the certified value should be an accurate estimate of the

true value with a reliable estimate of the uncertainty compatible with the end use requirements'.

Depending on the type of property value to be certified and the type of CRM, there may be differences in the approach applied. Matrix CRMs cannot be certified on the basis of direct mass determinations of constituents. Calculable (primary) and relative (rarely comparative) methods have to be used often after a total transformation or removal of the matrix. For such matrix CRMs there are three possible approaches:

- certification within one laboratory using one so-called definitive method by two or more independent analysts;
- certification within one laboratory using two or more so-called 'reference' methods (not primary) by two or more independent analysts;
- certification by interlaboratory studies using one or several different methods including (possibly) primary methods.

In all cases, only laboratories of the highest and proven quality should be involved.

5.1.4.1. Single laboratory approach

The first two approaches using definitive or primary or reference methods within one single laboratory require that in this laboratory everything is done to eliminate sources of systematic errors. Experience has demonstrated that it is very difficult to achieve 100% certainty and that within the laboratory a systematic bias does not remain. An additional confirmation through an — even limited — interlaboratory study is therefore advisable. Such an approach is used by NIST; the single laboratory certification complies with the demand of US law that results and certificates must be 'NIST traceable'.

5.1.4.1.1. Definitive methods / primary methods

For some chemical parameters so called direct methods, which need no external calibration, e.g. gravimetry, titrimetry, volumetry, coulometry, or definitive methods exist. Isotope dilution mass spectrometry (IDMS) with electrothermal ionisation as developed for nuclear measurements in the early fifties [4] can be a definitive method and has been adapted to the non-nuclear area [5,6]. Under certain conditions, such as total matrix destruction, separation of the analyte and optimal isotopic abundance ratios etc., only some elements e.g. Li, Na, Cu, Cr, Fe, B, Pb, Cd, Rb, Se, Ca, Si, Ba, Ni etc., which are often present as traces in biological or organic matrices, can be determined by this technique but only in some very few highly reliable laboratories. Unfortunately, the thermal ionisation system, which provides the most accurate and precise determinations, is hardly available for highly volatile elements and those elements that are difficult to thermo-ionise (e.g. Pt). Some elements e.g. U, Si, C etc. can be determined in gaseous forms. ICP-MS with isotope dilution is becoming available since ICP mass spectrometers gain in mass resolution. Mononuclidic elements are rarely determined by IDMS, as artificial isotopes would be necessary. Laboratories applying IDMS or other primary methods for certifications have developed their own procedure to calculate the uncertainty of the certified value. As such laboratories are metrologically highly reliable, this uncertainty is usually based on a complete uncertainty budget and calculated in accordance to the relevant ISO Guides [1,7]. NIST certifies materials

on the basis of own in-house produced data. The certified value is calculated through a weighted average of the measured values and a weighted estimate of the uncertainty [8].

Certification of matrix materials through one single definitive method (e.g. IDMS for pluri-isotopic elements) produces materials of high added value. Such CRMs do not give the user, who does not apply this method in daily practice, an indication of what can be achieved by other methods. They do not have a pedagogic role. As the use of CRMs is limited, this training aspect is still important. BCR mainly produces CRMs as a result of research projects and makes them available with the information gained during their development. The user can find information on the performance of a method similar to the one he uses and may also get a fair estimate of the uncertainty achievable by more classical methods. This can only be achieved with the certification through several methods within interlaboratory studies. For many fields of chemical measurements definitive methods do not exist. These are very limited in their field of application at the level of matrix and property values to be certified. They do not exist yet for the certification of traces of organic or organometallic compounds nor for the determination of mono-isotopic elements, or the determination of forms of elements with different oxidation states. For all these parameters, only an interlaboratory certification study with several different validated methods may be applied.

5.1.4.2. Interlaboratory certification study

Interlaboratory certification studies are organised following the same basic principles as other interlaboratory studies (see Chapter 12) but involve only highly specialised laboratories. They must follow strict rules so that all the analytical information on the measurements performed can be traced back and verified. All participants must have demonstrated their ability to perform reliable measurements in prior exercises: degree of accuracy achieved for the determination of the property value to be certified in the given matrix, internal quality control, validation of the method including evidence of statistical control, etc. The organiser of the study has also to fulfil many requirements and should be known and recognised for its ability to organise such studies. The best way to establish the reliability of all participants involved is to ask them to demonstrate their performance in so-called step-by-step improvement schemes as described in Chapter 12. This approach has been used by the BCR for all RMs where new property values were certified for the first time in matrix materials. Some of these step-by-step schemes have taken several years before a material could be certified (e.g. 8 years for dioxins, 4 years for PCBs and As species, two years for PAHs, etc.).

5.1.4.3. The BCR approach

Besides the general rules given above, particular and severe requirements underpin the BCR certification studies. In all cases, detailed protocols and reporting forms are prepared, requesting each participant to demonstrate the quality of the measurements performed, in particular the validity of calibration (including calibration of balances, volumetric glassware and other tools of relevance, use of calibrants of adequate purity

and known stoichiometry, proper solvents and reagents). Such protocols are the result of the preliminary feasibility studies (improvement schemes). The absence of contamination has also to be proven by procedure blanks. Where necessary, chemical reaction yields should be known accurately and demonstrated. All precautions should be taken to avoid losses, basic sources of errors like those listed in the section on method validation should be checked (e.g. formation of insoluble or volatile compounds, incomplete extraction and clean-up, etc.). If the results of entirely independent methods such as IDMS, AAS, voltammetry, INAA (between-method bias) for inorganic trace determinations as applied in different laboratories working independently (between-laboratory bias) are in agreement, it can be concluded that the bias of each method is negligible. As a consequence, the mean value of the results is the best approximation of the true value. Examples of protocol and reporting forms can be found in Annex 5.1 and 5.2.

5.1.4.3.1. Technical examination of data

As interlaboratory studies imply that data are generated in various locations, it is necessary to bring the data producers together so that they can examine and judge the value of the measurements. Before any further treatment, all data used to certify the material must be validated by the certification group. Therefore, they must first be collected by the organiser, and presented in a way in which they can be easily compared. Statistical representations may be used to highlight trends or systematic errors within or between sets of data.

In cases where methods are similar or show common steps, it is essential to examine if no systematic bias is affecting the results. In some few cases, BCR was confronted with situations where participants had to use a common calibration material (certification of dioxins and furans in a fly ash extract). Such a situation offers the possibility of introducing a systematic error into the study and consequently of a bias of the certified value. Situations where suppliers cannot propose reliable calibration substances may oblige the certification body to provide a common material. It should remain an exception and if unavoidable, the producer of the common calibrant should follow a strict preparation procedure similar to the one described above for the certification of calibrants.

Before examining the results, it is common practice in BCR certification studies to examine the methods applied. For method dependent parameters, e.g. in microbiology, the data are only disclosed to the participants after the examination of the full follow up of the protocols. Besides quality control steps and verification of calibration materials, e.g. with a common verification solution, each method is discussed to evaluate if inadequate handling was performed or risky steps applied.

For organic and organo-metallic substances, extraction efficiency (recovery) studies are performed to correct for eventual losses. Recovery figures can be obtained from standard addition procedures or successive extractions of the material. Standard additions techniques give the extraction efficiency through the calculation of the slope of the standard addition regression line. The uncertainty of the recovery figure can also be calculated directly from this regression line [9,10]. Multiple extractions are mainly used for solid matrices which already contain high amounts of the substance of interest

(highly contaminated materials e.g. industrial soils, solid waste). This makes the standard additions difficult as very high quantities have to be added which may not be compatible with the analytical method (saturation of system). The successive extractions of the sample can be performed with the same or different solvents or mixtures of solvents. At each step the remaining substance is measured. The sum of all extracted amounts gives the 100% figure and the extraction efficiency of the first step is the corresponding proportion of the total. The analyst must also estimate the losses occurring in the purification step. The uncertainty of the recovery figure is obtained from the sum of the uncertainties of all steps and of the limit of detection in the last step. No system to establish the recovery figure is generally applicable and all systems suffer criticisms. Nevertheless, they are absolutely necessary to certify the true content of a substance in a material. Only recovery figures that show a sufficient reproducibility can be used to correct the results. An acceptable reproducibility is similar to the reproducibility of a same number of independent measurements. Too low extraction efficiencies reflect poor extraction procedures and should be discarded. Clean up steps should not add significantly to uncertainty. Many purification procedures are available; therefore, any significant (measurable) loss due to clean up should lead to a change of the procedure.

Procedure blanks are performed at each occasion of measurement and their results are discussed before correction can be applied. Finally, all results are corrected for the water content in the sample. Particular attention is given to the reliability of the signal of the detector and the calibration of this signal. For detectors with a poor linear dynamic range the calibration must be performed in an adequate concentration so that extrapolation is possible to the signal of the unknown sample. Finally, all chromatograms — samples with and without internal standard(s), blanks, calibration solutions — are examined by all participants collectively. Any remaining unresolved question or any doubt on one or the other step in a procedure that may affect the trueness of the result leads to rejection of the data. The methods and data that successfully passed this technical evaluation are used to calculate the certified value and its uncertainty.

Trueness of the certified value will depend on this technical evaluation of data. Any doubt on the reliability in terms of trueness of a procedure should lead to rejection of the corresponding set of data. The precision of the sets of data will affect the uncertainty of the certified value. This uncertainty will reflect the state of the art for the certified parameter in the given matrix. As such it does not affect systematically the analytical value of the CRM. The participants in the certification campaign must also give their opinion on the overall value of the CRM and answer the following questions:

- Is (are) the certified value(s) reliable?
- Is (are) the certified value(s) sufficiently precise?
- Is the material still useful, e.g. are the certified parameters adequate, sufficient in number, fulfilling the requested task (e.g. regulatory purposes)?
- Is the material of any value for the end-users (confirmation of potential market)?

When finishing the technical evaluation of the data, a statistical treatment is performed to confirm the absence of outlying mean values, to examine the precision of the sets of data and to calculate the certified value and its uncertainty.

Consequently, it seems clear that a value obtained through simple interlaboratory studies or proficiency tests should never be accepted as certified. Such studies do not

select the laboratories *a priori* and do not foresee all the quality control points listed above to verify the validity of the produced data. Certification through interlaboratory studies does not just consist in calculating a 'consensus value' from uncontrolled data whatever way of calculating the assessed value is used.

5.1.4.3.2. Statistical examination of data

Statistics offer a large panel of tests to examine the data generated by the laboratories and to calculate the certified value and its uncertainty. BCR uses an unweighted method. The statistical treatment examines two parameters:

- the mean values reported by the laboratories and their distribution;
- the distribution of the variances of the reported sets of data.

The evaluation of the distribution of means is performed through a Dixon test (Nalimov). If outlying mean values remain after the technical discussion, it demonstrates that biased results remain and that this technical examination was unreliable. The parameter cannot be certified as a doubt remains on the trueness of the data.

Outlying variances, e.g. as reflected by a Cochran test, do reveal that some sets of data suffer insufficient precision compared to the other sets obtained by other laboratories. Such sets affect the final uncertainty of the certified value but not the certified value as such. The technical discussion should address the reason why a set of data lacks precision (day to day bias?) or why in one laboratory the reproducibility figure is much lower than for the rest of the participants (repeatability figure rather than reproducibility, selection of data, not fully independent measurements? etc.). Sets of data are rejected if the standard error of the mean (s/n) exceeds the standard deviation of the distribution of all the laboratory mean values. It must be stressed that BCR has accepted and even promoted alternative methods of measurements in some certification exercises, in order to back-up trueness of certified values. Finally, such methods may have shown that their precision was too poor and were not used to calculate the certified value and its uncertainty. In such cases the results are made available to the user of the CRM through the certification report.

Additional characterisation tests are performed to examine the population of data. They do not lead to decisions on whether or not a parameters should be certified or a set of data should be excluded e.g. normality of distribution of means and individual data (Kolmogorov-Smirnov-Lilliefors), consistency between laboratories of variances (Bartlett), etc. Many other tests could be performed before calculating the certified value. No definitive rules are given in the various guides of ISO [1,7]. The basic principle should remain as follows:

- no cosmetic statistics should be applied, i.e. complex statistics to give the impression that the system is scientifically sound, or statistics to hide poor results;
- the final word should always remain with the analytical chemist or biologist and not with the statistician, i.e. the analyst is the only one who can judge if the final result of the uncertainty is still of interest for the end users.

Table 5.1 shows the summary outcome of some parameters of certified values obtained for dioxins in milk powder and Figure 5.1 to 5.4 the corresponding graphical presentation.

TABLE 5.1

SUMMARY OF STATISTICAL RESULTS FOR TWO PCDD AND TWO PCDF IN BCR-CRM 529 (RAW DATA)

Certified property	D 48	D 66	F 83	F118
Number of data sets	12	9	7	9
Number of individual data	60	45	35	45
Compatibility of data sets two by two — Scheffe's multiple t-test ⁽¹⁾	12/66	8/36	2/21	8/36
Outlying data sets (Dixon, Nalimov tests) ⁽¹⁾	NO	NO	NO	NO
Outlying variances (Cochran test) ⁽¹⁾	YES	NO	NO	YES
Mean of means of data sets (ng/kg)	4499.1	1216.5	78.09	3396.0
Within data sets standard deviation (ng/kg)	381.1	121.15	9.36	259.6
Between data sets standard deviation (ng/kg)	819.4	267.22	12.98	585.3
Homogeneity of variance (Bartlett test) ⁽¹⁾	NO	YES	YES	(2)
Standard deviation of the data set means (ng/kg)	836.9	272.7	13.63	596.7
Normality of the distribution of the data set of means (Kolmogorov-Smirnov-Lilliefors test) ⁽¹⁾	YES	YES	YES	YES
Range of mean values (lowest)	3046	729	60	2588
(highest)	5904	1684	102	4525
Half-width of the 95% confidence interval (ng/kg)	531.7	209.6	12.61	458.7

⁽¹⁾: tests performed at the 0.05 and 0.01 significance levels ⁽²⁾: yes at 0.01 not at 0.05 significance level

5.1.4.3.3. Calculation of the certified value and uncertainty of matrix materials

The only additional attention to be given to the calculation of the certified value and its overall uncertainty is the existence of additional sources of uncertainties, which are not covered by the interlaboratory study. As discussed above, the inhomogeneity of the material may be such an additional source of uncertainty. Usually, materials brought by BCR to the final stage of certification do not suffer measurable heterogeneity. The systematic distribution to each participant (often between 15 to 20 laboratories) of two vials of the CRM and the obligation to perform the measurements on both samples introduces into the within- and the between-laboratory variance a possible uncertainty due to heterogeneity. An analysis in the form of a two-stage nested design could overcome the question. Experience shows also that the 95% confidence interval of the certified value is usually larger than the variation measured in the between-vial homogeneity test.

As mentioned and discussed in the section on stability testing, no uncertainty factor should come from a possible instability. It is far too hazardous to try to deduce from simple measurements over a certain period of time, which type of decrease a possible degradation of the material will follow. Degradation, as discussed in Chapter 4, can affect the matrix and the substance certified. The kinetics of the degradations are not simply of a first order and may even change in time. Therefore, corrections of any kind for instability should be forbidden. *Unstable materials should never be certified.*

Lab	REPLICATES					MEAN	S.D.
01	4257	4247	4106	4106	4136	4170	76
02	4875	4683	4557	4515	5351	4796	340
03	5383	5707	5292	5409	5165	5391	201
04	4410	4560	4610	4300	4600	4496	136
06	4379	5709	5619	5097	5681	5297	571
07	5630	5187	4803	3504	4085	4642	852
08	3054	3427	3839	3135	3315	3354	308
10	6147	6063	6104	5745	5462	5904	294
11	2715	3011	3081	2928	3496	3046	287
12	4730	5093	4519	4277	5052	4734	348
13	4359	4334	4471	4469	4534	4433	84
15	4090	3374	3509	4101	3551	3725	345

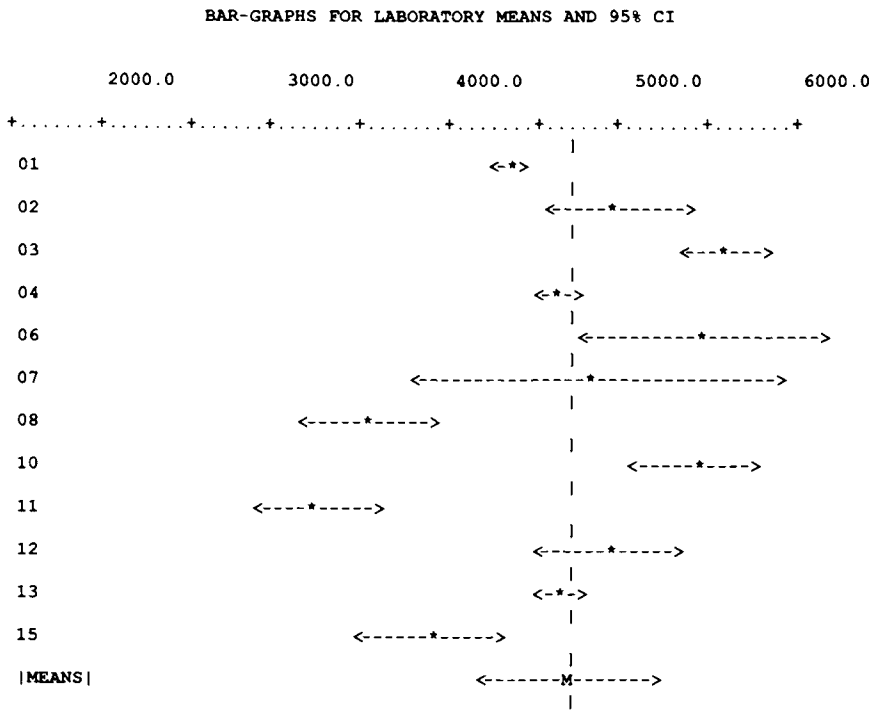


Fig. 5.1. Table of results and graphical presentation for 2,3,7,8-TCDD (D 48) in BCR-CRM 529 expressed in ng kg⁻¹ [49]

The discussion on remaining sources of uncertainty which have arisen recently are the addition of an uncertainty component to the final certified value due to the precision of the recovery factor used to correct for extraction efficiency and purification [9] and somewhat the correction to dry mass. This debate is far from closed. It may be considered that the uncertainty of the recovery factor is reflected in the variability of the pretreatment procedure of the certification measurements. This is true if this uncertainty of the recovery factor as determined by e.g. spiking or multiple extraction procedures shows reproducibility close to the certification measurements. If not, as discussed above, the

Lab	REPLICATES				MEAN	S.D.
01	1157	1137	1117	1087	1017	1103
02	1112	1174	1193	1141	1515	1227
03	1828	1729	1621	1685	1561	1684
04	1390	1680	1430	1560	1180	1448
07	1335	1178	1400	1049	1149	1222
08	1420	1299	1282	1408	1433	1368
12	766	641	626	781	831	729
13	1101	1152	1317	1152	1090	1162
15	956	851	1108	1141	964	1004

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

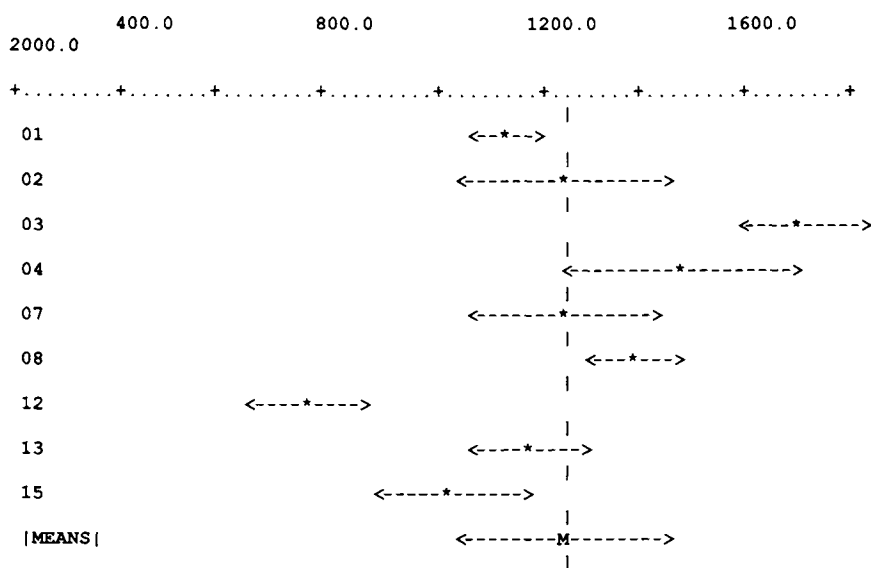


Fig. 5.2. Table of results and graphical presentation for 1,2,3,4,7,8-HxCDD (D 66) in BCR-CRM 529 expressed in ng kg⁻¹ [49]

data should not be used at all for the calculation of the certified value. The same holds for the determination of the water content. Too many variations show unreliable performance. Recently, BCR has started the certification of substances in fresh materials, where the result is expressed on the mass of material as received — i.e. wet basis [11].

As a final result the producer issues a material certified for a number of parameters, based on the minimum sample size determined in the homogeneity study. This information must be given to the end user in the form of a certificate. The certificate or the accompanying report must also state the uncertainty and the way it is calculated.

To calculate the certified value, BCR uses the sets of data delivered by the participants in the certification study. Usually 5 to 6 (up to 10 in special circumstances) taken at

Lab	REPLICATES				MEAN	S.D.
02	72.9	77.4	76.1	75.1	84.8	77.3
03	56.4	64.4	66.2	67.7	69.9	64.9
04	50.0	70.0	60.0	60.0	60.0	60.0
07	90.6	73.3	84.2	62.0	88.0	79.6
10	94.7	112.7	113.5	101.9	87.6	102.1
13	89.9	93.4	96.5	70.8	69.9	84.1
15	85.9	69.3	90.9	73.2	73.8	78.6

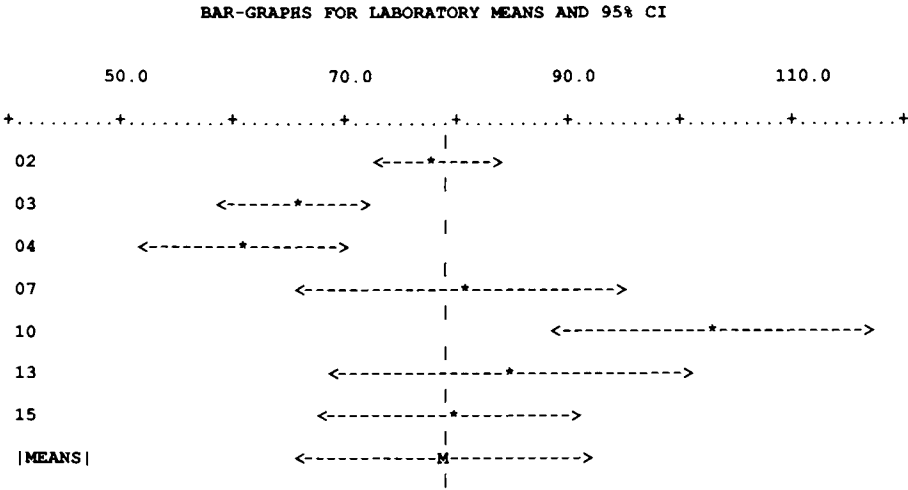


Fig. 5.3. Table of results and graphical presentation for 2,3,7,8-TCDF (F 83)in BCR-CRM 529 expressed in ng kg^{-1} [49]

least from two sets of sample are collected. They all have to have fulfilled the requested criteria laid down in the study protocol. They all have to pass the technical screening in a meeting where all participants have to be present. The certified value is calculated as the mean of the laboratory means. As different methods are used (to minimise systematic errors) by laboratories working independently, the individual data are not pooled. Pooling is done only when a material is certified through a given method. After having applied a Dixon (Nalimov) test on the sets of data issued from the technical screening, the mean of means is calculated. If the Dixon (Nalimov) test detects an outlier of mean the parameter is not certified. Otherwise the mean of mean will represent the certified value. The uncertainty is obtained as the 95% confidence interval of the sets of data — after removal of outliers of variance as discussed in section 5.1.4.4.2. This uncertainty may be corrected for additional components, e.g. heterogeneity, as discussed in section 4.3.3. Other expressions and approaches exist, in particular for the expression and calculation of the uncertainty. They all have to fulfil the basic principles set by the GUM [7]. This Guide is based mainly on the experience and examples taken from physical metrology and is written in general terms. Therefore, it is often difficult

Lab	REPLICATES				MEAN	S.D.
01	3200	2858	3050	2939	2858	2981
02	3030	3057	2971	3043	3627	3146
04	3650	3480	3610	3390	3690	3564
07	4008	3537	3869	2705	3263	3476
08	2913	2885	3073	3282	3191	3069
10	4682	4509	4539	4544	4352	4525
11	2277	2266	2955	2484	2959	2588
12	4157	4308	3915	4157	3885	4084
13	2900	3018	3337	3201	3198	3131

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

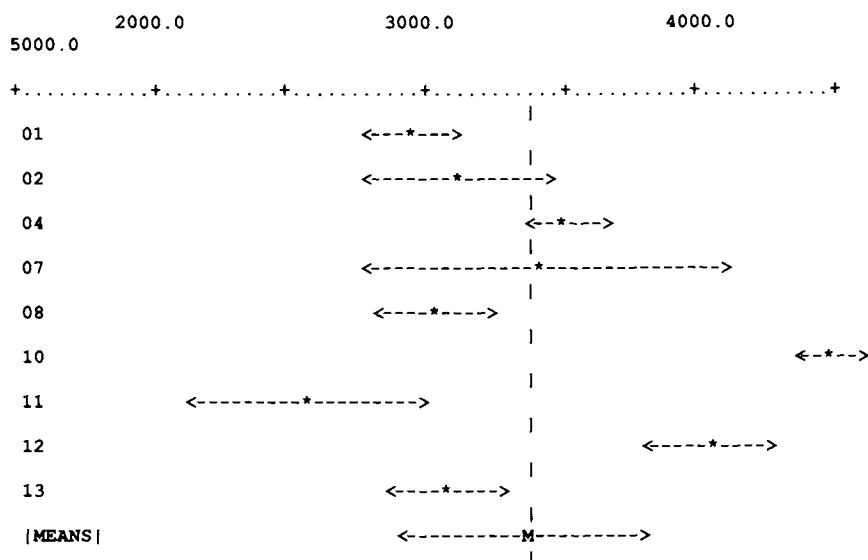


Fig. 5.4. Table of results and graphical presentation for 1,2,3,4,7,8-HxCDF (F 118) in BCR-CRM 529 expressed in ng kg^{-1} [49]

for chemists or biologists to interpret and adapt them to their specific situation. It is expected that in future specific actions will be taken in order to make explicit or 'translate' the GUM recommendations to fields such as organic, organometallic or biochemical trace analysis. A first step has already been made by EURACHEM in 1995 but would still need to be implemented further [12].

5.2. METHOD-DEPENDENT PARAMETERS

Analysts are increasingly confronted with measurements of parameters that do not represent the entire amount of a substance or element present in a matrix. These

fractions of the total content may correspond to a chemical form of an element, to a physical status in the matrix or to a fraction expressing a given property or activity, e.g. absorbed or adsorbed or dissolvable fractions. The fraction defined by the activity expressed by the substance could also be a toxic fraction, an enzymatic activity, etc. The latter is still not frequent in environmental monitoring but frequent in medical biology.

5.2.1. Chemical status

The analyst is often confronted with the determination of a certain chemical form of a substance or element, e.g. a particular isomer (optical isomers) or valence of an element. In this case the entire procedure consists in keeping this valence or chemical form. This is usually the case in organic and organo-metallic analysis (speciation). In some cases a particular method, or steps of a method, must be used which also defines the measured fraction.

5.2.2. Physical status

As already mentioned several times, the entire amount of a chemical substance is not always linked to the matrix in the same manner. It can be bound through different physical or physico-chemical liaisons. They may even be fully caught in the matrix structure and not available to extractants or digestion products. When applying drastic digestions or fusions, elements can be fully mobilised, but organic or organo-metallic substances are destroyed. Therefore, in certain cases the pretreatment will segregate the fractions of interest, e.g. selective extractions, enzymatic attacks etc. The analyst must link his result to the pretreatment procedure. In recent years, studies have been performed on pretreatment methods that mobilise some fractions only of the elements or substances [13–15]. Such an approach has been used to evaluate which fraction of elements can be displaced in nature by natural reagents e.g. organic acids in soils etc. They could also be used to evaluate the physiological activity in an animal or a plant organism (release of toxic substances stored in fatty tissues). When a certification of such fractions is pursued, the laboratories have to apply a strict analytical protocol. An essential part of the evaluation of the results consists in the verification that the protocol has effectively been followed. The certified parameter will be linked to the analytical procedure. Similar situations may be encountered when a standardised method leads to certification of a material.

5.2.3. Activity linked fractions

Similarly to the category of materials certified for some fractions of materials, one can certify a material for its activity. This is the case for enzymatic activity. The catalytic activity is certified on the basis of a standardised method (e.g. WHO method). Such activities are measured on a defined substrate e.g. kinetic of transformation of the substrate [16]. The biomedical field is the most active in the certification of such

parameters. In the near future the production of CRMs for other enzymatic activities, immunological reactions or vaccine activity should increase.

5.2.4. CRMs for testing

CRMs are materials that are developed for the determination of quantities of substances. One of their primary qualities is that, before quantification, they also allow the identification of the substance to be measured. CRMs have been produced for which the measurement aspect does not have to be performed. They are only certified for the presence or absence of a parameter. This is the case for the low contaminated microbial reference materials used for the validation of presence-absence tests in microbiology [17,18]. Similar materials are under study in other fields by BCR, for the detection of genetically modified organisms. They are still certified (for their identity) reference (because they serve as reference) materials. They represent as such another sort of materials, which enlarges greatly the field of application of CRMs. They should find applications in the validation of testing activities, screening methods and mainly biological sciences.

5.3. CERTIFICATION OF CRMS FOR MICROBIOLOGY

The first material certified for a living organism appeared in 1994 [17,19]. Since then several others have been added by BCR [17,20–22]. They all proceeded from the same approach: bringing a living organism into a survival stage which avoids total death or multiplication and which still allows a representative behaviour of the microbe when placed back into favourable living conditions.

5.3.1. Types of materials

BCR reference materials (certified or not) are composed of microbes stabilised in spray-dried milk powder. They have been produced from aged populations of representative strains of microbes. These materials are considered as representative and useful matrix CRMs for the examination of methods for milk monitoring. In fact they are also used for monitoring the performance of methods for water and various food matrices. The developed materials are used as materials to be added to the matrix to be studied (e.g. added into water, minced meat, etc.). These 'spiking materials' present two levels of contamination. Low level materials serve for verifying testing methods (yes/no answer). High level contaminated materials serve to validate methods used to count the number of germs present in a material. Figure 5.5 shows the differences between these two types of CRM. Other non-certified materials have been developed by SVM as a result of studies financed by BCR. Other producers have also issued RMs which have not yet been certified. Lightfoot and co-workers have developed lenticules of bacteria stabilised by drying in a protective matrix, which is adapted to each strain. Numbers of strains and mixtures of strains are under study and tested in large-scale proficiency tests [23,24]. This new approach seems to lead to materials that are easier

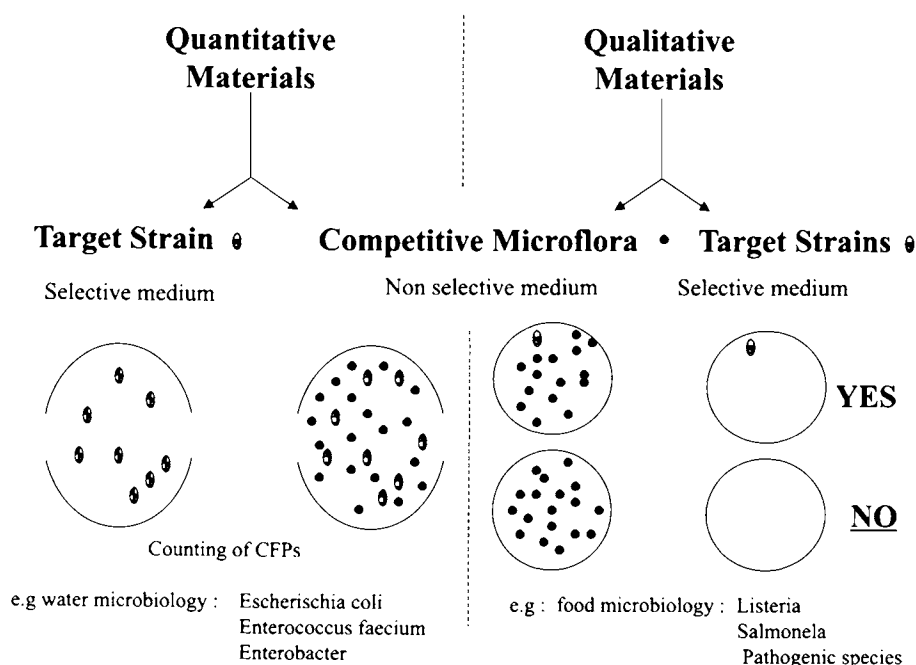


Fig. 5.5. High level contaminated reference materials (left) lead to counting of colonies in a dish. They are mainly used to validate methods following indicator organisms. Low level contaminated materials serve to validate yes/no (testing) methods. They are applied for the monitoring of pathogens.

to handle than the milk powder capsules of BCR. Other freeze-dried RMs have been developed by the National Food Administration in Sweden [25]; the samples are stored at -20°C . Other non-certified materials appear regularly in proficiency testing schemes. None of these materials have been certified. For all RMs and CRMs produced so far, one of the major difficulties is to remain with a representative strain when the material is stabilised i.e. it behaves in a similar way to 'wild strains' encountered in nature when used with a given method of measurements or test.

5.3.2. Stabilisation of microbes

It would be too tedious to go into details of the production of BCR CRMs for microbiological testing. The studies and detailed procedures have been published elsewhere [26]. As already mentioned the selected strains are first stabilised in spray-dried milk powder by a long storage at cool temperatures (-20°C) for sometimes several years. Then a fraction of this mother batch is mixed with sterile milk powder down to a level of contamination close to the target level. Usually, after the preparation of the diluted milk powder, a destabilisation of the population is noticed and the material is left for a longer period at low temperatures to reach again a stable contamination level. After

verification of the homogeneity, this 'diluted milk powder' is filled into gelatine capsules. Figure 5.6 shows the simplified production procedure. For some strains it has been demonstrated that the composition of the milk powder is of great importance. The addition of sugars, e.g. sucrose, can improve the survival and stability of a strain [27,28].

5.3.3. Homogeneity tests

The distribution of a limited number of microbes within a solid matrix is essentially inhomogeneous, i.e. for a certain level of intake, differences due to the distribution of the microbes will appear. The more microbes that have been introduced into the matrix, the less these differences will be important and the lower the sample intake will be where differences appear. The theoretical distribution of the microbes in the sample is described by a Poisson distribution [29,30]. The RIVM group, which developed these materials, used a modified Cochran's dispersion test to evaluate the variation within a single capsule and between capsules. In fact overdispersion (more inhomogeneity than theoretically expected) has been noticed for nearly all RMs and CRMs produced [31]. The certification trial revealed that it had no influence on the outcome of the interlaboratory certification study as the between-laboratory reproducibility largely covered this overdispersion factor.

5.3.4. Stability tests

As already mentioned, stability of microbes is the limiting factor for CRM production. Microbes are living organisms. They can die or multiply (at least the dead ones are of no interest!). Between both extremes, they can be biologically more or less active. Activity is often related to potential pathogenicity. Selecting the 'bad ones' is the objective of the microbiologist. Therefore, his method will be more or less selective. The more selective a method (culture medium and conditions), the less will injured bacteria form colonies and be counted. This selectivity aspect also reflects the difficulty of producing CRMs for microbiology. The objective is to avoid multiplication of the microbes as well as their death. Therefore, they must be brought into a survival stage. Put back into favourable conditions the microbes must multiply again to form colonies like in natural conditions. This must be repeatable and 'accurate' in microbiological terms i.e. the same number of colony-forming particles (cfp) under similar culturing conditions. When culturing conditions change — composition of the medium, temperature, duration, etc. — the formation of visible cultures will also change. The count is method-dependent. Equally, the control of the stability of the CRM will be method-dependent. Once this is achieved some form of traceability to a known identified strain is achieved. The stability of microbes in the matrix can be achieved, as was demonstrated by BCR, through procedures that can be found by those interested from the technical reports published by the European Commission and by RIVM [26]. Stability was achieved by combining the most recent knowledge of microbial biology and 'kitchen tricks' in the preparation of the survival medium. Finally, new statistical approaches were developed to estimate the stability of the CRM [31]. Figure 5.7a and 5.7b show the effect of temperature and storage time on the stability of *Salmonella thyphimurium*. At higher

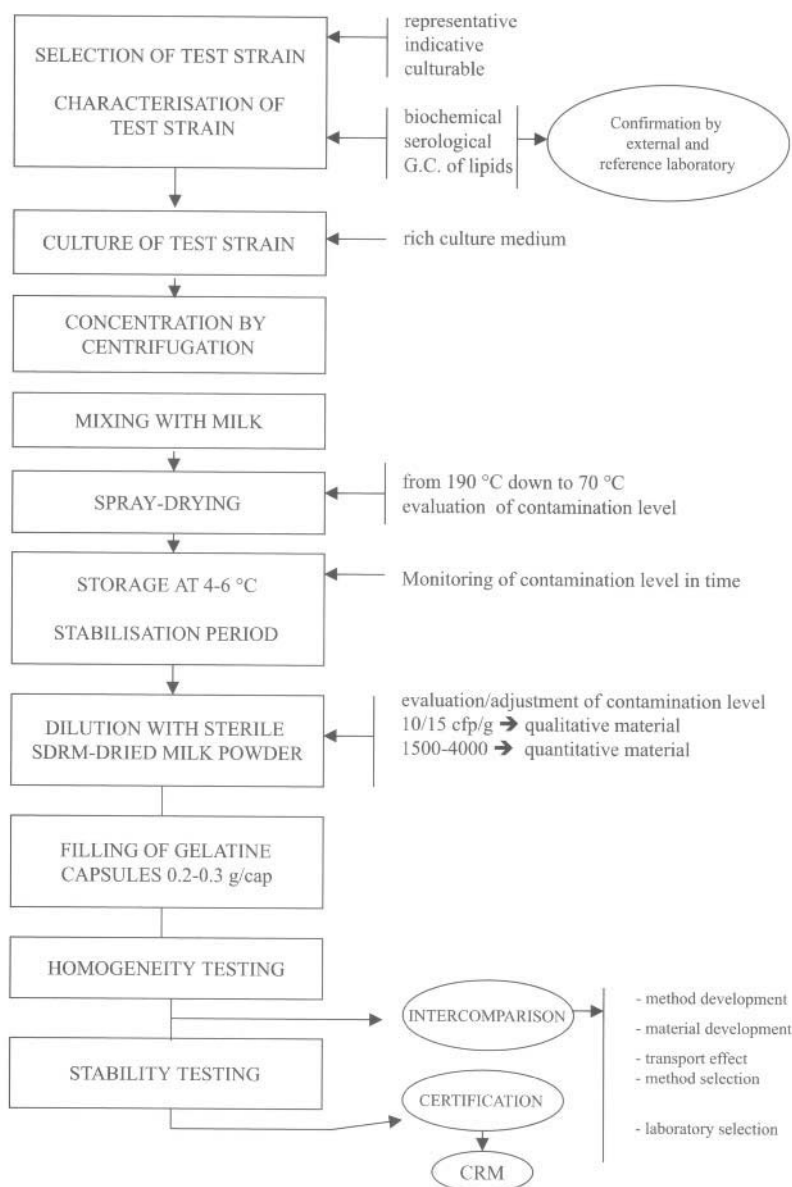


Fig. 5.6. Overall production scheme of BCR microbiological RMs and CRMs.

The stabilisation of microbial strains in spray-dried milk powder has allowed the preparation of several RM available from RIVM in the Netherlands and six BCR-CRMs. Some additional strains have demonstrated sufficient stability to be candidates for certification. Other types of materials could be or have been developed by various producers (see text). For certification purposes a similar approach to the one used by BCR could be used for these materials.

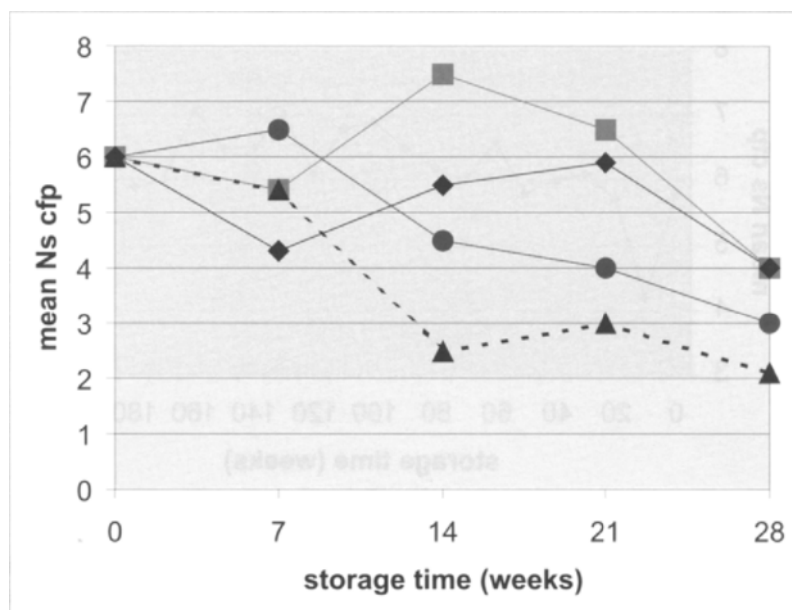


Fig. 5.7a. Stability study of *Salmonella typhimurium* (BCR-CRM 507) at various temperatures of storage.

At +37 °C the number of cfp decreases rapidly. Y axis: number of colony-forming particles of *Salmonella typhimurium* in one capsule (Ns.cfp). X axis: storage time in days. ■: -20 °C, ◆: +22 °C, ●: +30 °C, ▲: +37 °C.

temperature (30°C and more) the number of colony-forming particles decreases rapidly. At -20°C little change is noticed over 200 days.

5.3.5. Certification by interlaboratory studies

In principle, the certification of RMs for microbiological parameters does not differ from the approach used by BCR for chemical parameters. They fall into the category of method-dependent CRMs certified for an activity. In fact, the microbes will behave differently from one type of method (in particular the culture medium) to another. For selective media the 'resuscitation rate' of the microbes in the RM will change. Injured bacteria will have difficulties to form colonies on these media. This reflects the biological activity of the strain after stabilisation. As the method will strongly affect the result, it must be verified that the method was applied in a similar way by all participants. This means that accurate protocols have been prepared and tested beforehand in a feasibility study. The first step of the evaluation of the certification study will be to verify that the protocol was strictly followed. This protocol will be made available to the users of the CRM.

Such certifications are only of interest if the method(s) used are recognised by the scientific community as reference methods. Therefore, BCR has certified its RMs through

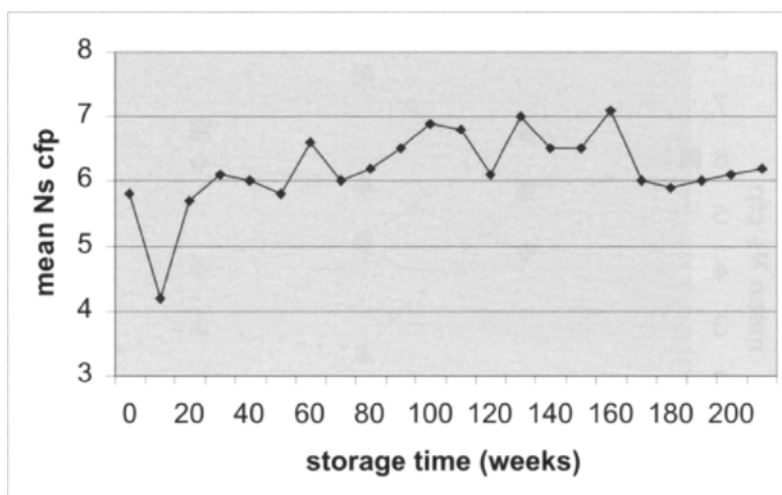


Fig. 5.7b. Long-term stability of *Salmonella typhimurium* (BCR-CRM 507).

No major decrease in cfp per capsule could be noticed at -20°C over 200 weeks. Y axis: mean number of colony-forming particles of *Salmonella typhimurium* in one capsule (Ns, cfp). X axis: weeks of storage at -20°C .

ISO or other widely used standard methods. Nowadays, it seems still difficult to certify parameters in microbiology without passing through specified methods. The certification of the number of colony-forming particles in high or low 'counts materials' are based on the properties of small numbers of discrete particles. Commonly applied normal distributions, as used in chemistry, cannot be used to handle data and estimated uncertainties as collected in microbiology. Theoretical and experimental considerations have shown that ideally the counts follow the well-known Poisson distribution, which is characterised by the variance being equal to mean. In many real cases encountered in the interlaboratory studies, the actual dispersion of data was greater than what was expected from a Poisson distribution. The statistical approach used for the certification of reference materials for microbiology have been discussed extensively and published in a report published by the BCR in 1993 [30]. In high count capsules e.g. *Enterococcus*, *Enterobacter*, *E. coli* and *Bacillus cereus* [19–22] the number of colony-forming particles in a capsule is certified. In low count capsules used to validate presence/absence tests of food pathogens, e.g. *Salmonella* and *Listeria* [17,18], the probability of detecting, through a given method, a capsule that does not contain a microbe is certified. For high counts the geometric mean with a 95% confidence interval of the results delivered in the interlaboratory certification study represents the certified value. For low count materials the proportion of 'zero' materials (capsules without bacteria) reported by the participants with a one sided 95 % confidence upper limit is taken as the certified value. In addition an attempt was successfully made to also certify the counts of bacteria in the low contaminated materials as in high counts.

Compared to physics or chemistry, microbiology is not yet able to measure absolute parameters (non method-dependent). Therefore, concepts like accuracy or traceability

are procedure dependent. On the other hand, microbiologists measure only those artefacts that are of pathological or biological relevance — dead bacteria are not of any interest. By achieving this, their analytical result already includes an answer for the end user and this is rarely the case in chemistry. Therefore, the poorer precision of produced results is balanced positively by the added value of the information delivered.

Microbiology, like other fields in biology — immunology, parasitology, genetics etc. — is still in an early stage of development of quality control tools of measurements. When considering the economic impact, health, environment and safety concerns, there is no doubt that in the coming years they will form the field where research and development efforts for new CRMs will focus.

5.4. CERTIFICATE AND CERTIFICATION REPORT

5.4.1. Information content

The certification of a property value in a material leads to a certified value, which is typically the mean of several determinations or the result of a metrologically valid preparation procedure, e.g. weighing. The confidence interval or uncertainty limits of this mean value are also determined. The two basic analytical parameters, mean and uncertainty, are included in a certificate of analysis. The presentation and the additional information, which should also be given in the certificate, are listed in the ISO Guide 31 [32] and cover in particular:

- administrative information on the producer and the materials;
- brief description of the material with main properties and its preparation;
- intended use of the materials;
- information for correct use and storage of the CRM;
- certified values and confidence limits;
- other non certified values (optional);
- analytical method(s) used for certification;
- identification of certifying institute(s);
- legal notices and signature of certifying body.

An example of a typical BCR certificate is given in Annex 5.3. Not all information useful to the user of the CRM can be given in a simple certificate. Therefore, some CRM producers, e.g. BCR, make the material available together with a certification report which details the information given in the certificate. In particular, such reports highlight the difficulties encountered in the certification and typical errors that may arise using the material. The existence of extensive certification reports from BCR underlines the prime objective of this institution, which is to improve quality and comparability of measurements within the European Union. The certification report is a didactic vehicle for the dissemination of good analytical practice. The certification report is also a way of demonstrating the transparency of the certification procedure.

The entire certification work described in the BCR certification reports is examined by an independent group of experts so that all possible sources of unacceptable practice are detected and eliminated. The group of experts has strong metrological background

knowledge as well as training in the specialised analytical practice concerning the CRM. This group gives the final recommendation whether or not the RM can be certified and confirms the usefulness of the CRM for the scientific community. Finally, based on their advice the European Commission certifies the material.

5.4.2. Responsibility of the producer

Producing certified reference materials is a task that gives an enormous responsibility to the producer. The CRMs issued are used by the entire analytical scientific community as references in their own quality control systems. The analysts will trust blindly the certified value and they may change their methods or way of working according to the results they get when analysing the CRM. Therefore, the impact of the CRM will be enormous. Any error in the certified value may induce or increase the mistakes and their consequences that the analyst wanted to avoid. A wrongly certified value misleads the analyst. As a consequence, the certification of reference materials has to be in the hands of scientifically trained people, aware of the disciplines necessary to achieve the certification i.e. statistics, organic, inorganic analytical chemistry, microbiology, biochemistry, etc. They must be independent of the technical work itself so that they are not influenced in their decision when it has to be decided whether or not to certify. When certified, the producer engages legally its responsibility (whatever statement he puts on the certificate!). This responsibility stops when the material enters the user's laboratory as it cannot be verified if the storage and use is properly done by the operator. The producer must at least give information on the proper storage conditions. The transport of the material is only partially the responsibility of the producer. If the material is sent out under conditions that have demonstrated that they do not affect the CRM (e.g. obtained from the stability study) but the carrier has not respected these conditions, then the responsibility is not engaged. However, most producers replace affected (e.g. broken bottles, leaching vials, etc.) samples. If necessary they include recording systems e.g. for temperature control.

5.5. PRODUCERS AND PRODUCTION SYSTEMS

5.5.1. Producers

Several producers provide the analytical community with a wide range of CRMs. It is difficult to establish an exhaustive list of materials produced so far. The ISO Council on Reference Materials (REMCO) publishes a directory for Reference Materials and is preparing a status report on the worldwide production of CRMs [33], which lists more than 1500 CRMs already existing or in production and having projected completions up to 2001. ISO/REMCO has identified 168 producers of RMs. Some additional compilations of existing CRMs in more specialised fields, e.g. marine monitoring, also exist e.g. NOAA Data base [34]. The major source of information on reference materials, however, is the COMAR Data Bank which is a joint enterprise between the Laboratoire National d'Essais (Paris, France), the Bundesanstalt für Materialforschung und -prüfung

(Berlin, Germany) and the National Physical Laboratory (Teddington, Great Britain). It can be consulted on the WEB sites of these organisations. The major producers and suppliers of CRM in the non-nuclear field, fulfilling the conditions for the production discussed in this chapter are NIST, BCR, and the NRCC (Canada). Others within National Metrology Institutes also develop and provide CRMs e.g. LGC in UK, NMI in the Netherlands, BAM in Germany. Often they provide pure substances or CRMs for physico-chemical testing, more rarely matrix CRMs for environmental monitoring. New producers enter the market or remain more specialised. A tentative list of matrix CRMs available for environment monitoring is given in Table 5.2. In the field of nuclear and isotopic measurements, several specialised institutions provide CRMs e.g. IAEA (the International Atomic Energy Agency in Vienna mainly provides materials for nuclear measurements but also supplies some RMs for non-nuclear analysis), IRMM of the European Union, and NIST together with the US Department of Energy New Brunswick Laboratory.

5.5.2. Production systems

The level of quality of the RMs and CRMs available on the market has also raised questions. Until now the analyst has only had incomplete information or his own experience for judging the level of quality of the RMs proposed by various suppliers (reputation of producers). In order to improve the quality of available information and with the objective to improve the quality of the production of RMs and CRMs, ISO has set up a working party for preparation of guidelines for the producers. This ISO Guide 34 [35] mainly gives guidance on how to interpret the ISO 25/EN 45000 [36,37] and ISO 9000 [38] standards for RM and CRM production. The technical requirements for the production will also follow those set up in ISO Guide 31 and 35 [1,32]. Some producers, e.g. NIST, have published reports in the scientific literature on how they implement the certification work [8]. BCR has issued guidelines for candidate consortia on how to prepare a project on the production of CRMs to be submitted in the Commission's call for proposals [39]. Similar guidelines exist for CRM project managers [40]. BCR (the Measurement and Testing research and development activity of the European Commission is in charge of the development and production and the Institute for Reference Materials and Measurements of the EC is in charge of the service to customers) is slowly entering its production activities into a quality system based on the ISO 9001 standard.

The beginning of the 21st century will probably see the start of a more structured and transparent way of producing RMs and CRMs. This will have a consequence on the legal status of CRMs for the demonstration of method reliability. The evolution will encounter the demands formulated by legislation or decision-makers.

5.6. DRIVING FORCES TO THE FUTURE

CRM producers are faced with an increasing demand for materials. The evolution of modern society towards more safety, more guarantees, better products and services, more and better proofs in conflict cases will strengthen the drive towards more reliable

TABLE 5.2

EXAMPLES OF SOME RECENT CRMS (END 1998) FOR THE QUALITY CONTROL OF MEASUREMENTS IN ENVIRONMENTAL MONITORING (NON NUCLEAR FIELD AND ONLY CERTIFIED MATRIX MATERIALS)

Type	CRM No	Certified parameter	Supplier	Reference
<i>Sediments</i>				
Estuary	CRM 277R	trace elements	BCR	51
Lake	CRM 280R	" "	"	"
River	CRM 320	" "	"	"
Lake	CRM 601	extractable elements	"	"
Harbour	CRM 424	TBT	"	"
Estuary	CRM 580	Hg and MeHg	"	"
River-harbour	CRM 535	PAH	"	"
" "	CRM 536	PCB	"	"
Antarctic	MURST-ISS-1	trace elements	BCR/ISS	"
Estuary	SRM 1646	" "	NIST	46
River	SRM 2704a	trace and major elements	"	"
"	SRM 1939a	PCB	"	"
Marine	SRM 1941a	PAH-OCP-PCB	"	"
Waterway	SRM 1944	BeP	"	"
Marine	HS 3-6	PAH	NRCC	49
"	CS-1	aroclor	"	"
Harbour	HS 1-2	PCB	"	"
"	PACS-1	TBT + trace elements	"	"
Marine	BCSS-1	trace and major elements	"	"
Estuary	MESS-1	"	"	"
"	BEST-1	Hg	"	"
Harbour	EC-1	PAH	NWRI	"
Lake	EC-2	CB-PAH	"	"
River	EC-3	"	"	"
"	HR 1	trace and major elements	"	"
Lake	WQB 1-2-3	trace elements	"	"
Stream	GBW07-309 to 07-312	trace and major elements	NRC	"
"	GDS 10-12	"	"	"
Marine	GBW 07313	"	"	"
River	GSD 9	"	"	"
"	GBW 08301	trace elements	"	"
Pond	NIES 2	trace and major elements	NIES	"
Stream	SARM 46,51,52	"	SABS	"

TABLE 5.2

CONTINUED

Type	CRM No	Certified parameter	Supplier	Reference
<i>Soils</i>				
Loam	CRM 141R	trace elements	BCR	51
Sandy	CRM 142R	" "	"	"
Amended	CRM 143R	" "	"	"
"	CRM 483-484	extractable elements	"	"
Calcareous	CRM 600	" "	"	"
Industrial	CRM 524	PAH-PCP	"	"
"	CRM 481	PCB	"	"
"	CRM 529-530	PCDD/F-CB-CP	"	"
San Joaquin	SRM 2709	trace elements	NIST	46
Montana	SRM 2710-2711	" "	"	"
No inf.	EPA SRS003-50	" "	US-EPA	49
Industrial	EPA SRS103-100	PAH	"	"
No Inf.	GBW 07401 to 07408	trace and major elements	NRC	"
Tibet (no inf.)	GBW 08302	" "	"	"
Farmland	GBW 08303	" "	"	"
Brown	GSS-1	" "	"	"
Desert	GSS-2	" "	"	"
Yellow-brown	GSS-3	" "	"	"
Yellow	GSS-4	" "	"	"
Yellow-red	GSS-5-6	" "	"	"
Laterite	GSS-7	" "	"	"
Loess	GSS-8	" "	"	"
Sediment rich	SARM 42	major elements	SABS	"
<i>Sewage sludge</i>				
Domestic	CRM 144R	trace elements	BCR	51
Mixed origin	CRM 145R	" "	"	"
Industrial	CRM 146R	" "	"	"
Mixed origin	CRM 392	PCB	"	"
" "	CRM 088	PAH	"	"
" "	CRM 597	Cr	"	"
" "	CRM 677	PCDD-PCDF	"	"
Separator sludge	EPA-SRS101-100	PAH	US-EPA	49
<i>Ashes and dust</i>				
Coal fly ash	CRM 038	trace elements	BCR	51
Incineration fly ash	CRM 176	" "	"	"
" " "	CRM 490	PCDD-PCDF	"	"
Fly ash on filters	CRM 128	trace elements	"	"

TABLE 5.2

CONTINUED

Type	CRM No	Certified parameter	Supplier	Reference
<i>Ashes and dust</i>				
Welding dust	CRM 545	CrVI and leachable Cr	"	"
Coal fly ash	SRM 1633b	trace elements	NIST	46
" " "	SRM 2689-91	major and trace elements	"	"
Urban dust	SRM 1648	trace elements	"	"
" "	SRM 1649a	PAH	"	"
Diesel particulate	SRM 1650a	"	"	"
Diesel part. extract	SRM 1975	"	"	"
Diesel particulate	SRM 2975	"	"	"
Fly ash	EPA-SRS 001-100	trace elements	US-EPA	49
Incineration ash	EPA-SRS 019-50	" "	"	"
" "	EPA-SRS 203-225	" "	"	"
Coal fly ash	GBW 08401-08402	" "	NRC	"
Vehicle exhaust part.	NIES 8	" "	NIES	"
<i>Waters</i>				
Fresh water	CRM 398	trace and major compounds	BCR	51
	CRM 399		"	"
Sea water	CRM 403	trace elements	"	"
" "	CRM 179	Hg	"	"
Estuary	CRM 505	" "	"	"
Ground water	CRM 609-610	" "	"	"
" "	CRM 611-612	bromide	"	"
" "	CRM 616-617	major elements	"	"
Simulated rain water	CRM 408-9	major compounds	"	"
Fresh water	CRM 479-480	nitrates	"	"
Fresh water lyoph.	CRM 606	polar pesticides	"	"
Milk powder*	CRM 506	Enterococcus faecium	"	"
" "	CRM 527	Enterobacter cloacae	"	"
" "	CRM 594	Escherichia coli	"	"
Water	SRM 1643d	trace elements	NIST	46
Natural water	SRM 1640	" "	"	"
Water	SRM 1641	Hg	"	"
Simulated rain water	SRM 2694b	major parameters	"	"
Near-shore sea water	CASS-2	trace elements	NRCC	49
Open ocean water	NASS-4	" "	"	"
Estuary water	SLEW-1	" "	"	"
River water	SLRS-2	" "	"	"
" "	ORMS-1	Hg	"	"

TABLE 5.2

CONTINUED

Type	CRM No	Certified parameter	Supplier	Reference
<i>Waters</i>				
Water	V-SMOW and SLAP	O & H stable isotope ratios	IAEA	"
Sea water	IAPSO	conductivity	OSI	"
<i>Waste</i>				
Mineral oils	CRM 420-449	PCB	BCR	51
Shale oil	SRM 1580	PAH-phenols	NIST	46
Motor and transformer oil	SRM 1581	aroclor	"	"
Crude oil	SRM 1582	PAH-phenols	"	"
<i>Gases</i>				
On Tenax	CRM 112-562	benzene, toluene m-xylene	BCR	51
Permeation tubes	SRM 1625-26	SO ₂	NIST	46
" "	SRM 1629a	NO ₂	"	"
Air	SRM 1658a-69b and 2764/2750-51	CH ₄ - C ₃ H ₈	"	"
"	SRM 1671-72	CO ₂	"	"
"	SRM 2607-10	CO ₂ - NO ₂	"	"
"	SRM 2656-2660	NO _x	"	"
"	SRM 2612-14	CO	"	"
Permeation tubes	GBW 08201	SO ₂	NRC	49
" "	GBW 08202	NO ₂	"	"
" "	GBW 08203	H ₂ S	"	"
" "	GBW 08204	NH ₃	"	"
" "	GBW 08205	Cl ₂	"	"
Air	GBW 08119	CH ₄	"	"
"	GBW 08120	CO ₂	"	"
"	GBW 08123	CH ₄	"	"
<i>Animal tissues</i>				
Mussel tissue	CRM 278	trace elements	BCR	51
Cod muscle	CRM 422	" "	"	"
Milk powder	CRM 063R 150-151	" "	"	"
Human hair	CRM 397	" "	"	"
Plankton	CRM 414	" "	"	"
Tuna fish tissue	CRM 627	forms of As	"	"
" " "	CRM 463-464	MeHg + Hg	"	"

TABLE 5.2

CONTINUED

Type	CRM No	Certified parameter	Supplier	Reference
<i>Animal tissues</i>				
Mussel tissue	CRM 477	TBT, DBT, MBT	"	"
Cod liver oil	CRM 349	PCB	"	"
Mackerel oil	CRM 350	PCB	"	"
Milk powder	CRM 450	PCB	"	"
" "	CRM 607	PCDD-PCDF	"	"
Pork fat	CRM 430	OCP	"	"
Cod liver oil	CRM 598	OCP	"	"
Milk powder	CRM 187-188	OCP	"	"
Animal feed	CRM 115	OCP	"	"
Coconut oil	CRM 458-459	PAH	"	"
Oyster tissue	SRM 1566b	trace elements	NIST	46
Milk powder	SRM 1549-8435	" "	"	"
Bovine serum	SRM 1598	" "	"	"
Cow blood	SRM 955b	Pb	"	"
Cod liver oil	SRM 1588	PCB+OCP	"	"
Mussel tissue	SRM 1974a	PAH	"	"
Human serum	SRM 1589a	aroclor	"	"
Whale blubber	SRM 1945	OCP-PCB	"	"
Mussel tissue	SRM 2974	OCP-PCB	"	"
Lobster hepato.	TORT-1	trace elements	NRCC	49
" "	LUTS-1	" "	"	"
Dogfish liver	DOLT-1	" "	"	"
Dogfish muscle	DORM-1	" "	"	"
Mussel tissue	MUS-1	Domoic acid	"	"
Fish tissue	EPA-SRS903	chlordane-OCP	US-EPA	"
Mussel tissue	GBW 08571	trace elements	NRC	"
Prawn	GBW 08572	" "	"	"
Sea bass tissue	NIES 11	Sn, TBT	NIES	"
Aquatic plants	CRM 060-061	trace elements	BCR	51
Aquatic plant	CRM 596	Cr	"	"
Marine	CRM 279	trace elements	"	"
Olive leaves	CRM 062	" "	"	"
Hay powder	CRM 129	" "	"	"
Rye grass	CRM 281	" "	"	"
White clover	CRM 402	" "	"	"
Lichens	CRM 482	" "	"	"
Spruce needles	CRM 101	nutrients and	"	"
Beech leaves	CRM 100	contaminants	"	"
Citrus leaves	SRM 1572	trace elements	NIST	46

TABLE 5.2

CONTINUED

Type	CRM No	Certified parameter		Supplier	Reference
<i>Plant tissues</i>					
Pine needles	SRM 1575	"	"	"	"
Apple leaves	SRM 1515	"	"	"	"
Peach leaves	SRM 1547	"	"	"	"
Spinach leaves	SRM 1570a	"	"	"	"
Tomato leaves	SRM 1573a	"	"	"	"
Vegetation	SRM 2695	fluoride		"	"
Sargasso seaweed	NIES-9	trace elements		NIES	49

*Abbreviations used in Table 5.2**Substances:*

BeP: Benzo(e)pyrene

CB: chlorobenzenes — CP: chlorophenols

MeHg: methyl mercury

OCP: organochlorine pesticides

PAH: polycyclic aromatic hydrocarbons.

PCDD-PCDF: polychlorodibenzo-p-dioxins and polychlorodibenzo-furans

PCP: pentachlorophenol

TBT, DBT, MBT: tri-, di-, mono-butyl tin

Miscellaneous:

part.: particulate matter milk powder *: capsules to be dissolved into water

hepato.: hepatopancreas Lyoph.: lyophilised

CRM producers:

ISS: Istituto Superiore de la Sanita, Rome, Italy

NIES: National Institute for Environmental Studies of Japan, Tsukuba, Japan

NRC: National Research Centre for CRM, Beijing, China

NWRI: National Water Research Institute, Burlington, Canada

OSI: Ocean Scientific International Ltd, Wormley, Surrey, UK

SABS: South Africa Bureau of Standards, Pretoria, RSA

US-EPA: United States Environmental Protection Agency, Cincinnati, USA

measurement and testing methods. To test the performance of these methods more and better adapted CRMs will be necessary. CRM producers, in particular those acting within public services, will have the duty to develop them. As it has been said several times in the sections above, many aspects of the actual situation have to be improved or at least clarified (or sometimes made understandable). Many rules or guides are still reserved and accessible to a minority. To fulfil the forthcoming demands, in particular in fields where metrology is still underdeveloped, new concepts must be developed. Research must be started to investigate ways to provide analysts with reliable materials and adapted rules to use them.

In order to anticipate the growing needs for such materials in the forthcoming years,

the European Commission has carried out a study comprising an inquiry carried out in 18 European countries and a workshop with European experts in the field, which were co-ordinated by the Association Pôle Environment Sud-Aquitaine (APESA) in Pau (France). The following section summarises the main results of the inquiry and discusses prospects for the use and production of reference materials [41].

5.6.1. Salient features of the inquiry

Around 1600 European laboratories responded to the questionnaire (Table 5.3). From the answers received in the different countries, it was clear that many laboratories are not familiar with the term CRM and that confusion exists between certified and non-certified reference materials, between matrix RMs and calibrants. Besides RMs and CRMs it also became clear that many laboratories were not aware of the existence and even the meaning of proficiency testing.

The majority of laboratories that responded to the questionnaire (34%) were public laboratories, followed by industry (24%) and private testing laboratories (23%). Environment was the largest field of analysis covered by the study (26%), followed by food and feed-stuffs (20%). Tables 5.3, 5.4 and 5.5 give details on the profile of the

TABLE 5.3

TYPES OF LABORATORIES WHICH RESPONDED TO THE INQUIRY ON CRM NEEDS

Country	Number of quest. received	Types					
		Public	Private	Other	Industry	Research	University
Austria	38	6	9	2	12	7	9
Belgium	157	23	48	5	74	14	14
Czech Rep.	106	42	27	14	19	20	19
Denmark	34	18	13	0	3	9	1
Finland	67	42	12	1	9	27	1
France	108	59	27	6	19	14	6
Germany	113	23	29	7	4	19	47
Greece	87	8	11	6	31	15	24
Hungary	70	26	12	14	13	9	1
Ireland	71	28	12	3	16	6	11
Italy	71	35	7	0	8	7	14
Netherlands	131	37	26	26	33	23	3
Norway	41	18	11	1	4	13	0
Portugal	94	25	30	14	36	19	4
Spain	120	49	26	0	18	22	22
Sweden	117	37	32	4	45	18	4
Switzerland	94	48	20	2	13	13	15
United Kingdom	91	12	19	3	39	12	11
Total	1610	536	371	108	396	267	206

TABLE 5.4

FIELD OF ANALYSIS OF PARTICIPANTS IN THE INQUIRY ON CRM AND COUNTRY OF ORIGIN

Country	Environ	Food	Agricul.	Health/ Safety	Chemical	Ores/ Mineral	Fuel	Polymer	Pure metals	Other indust. prod.
AT	21	11	9	14	8	0	5	2	2	9
BE	57	29	21	37	41	7	7	37	5	41
CZ	84	46	35	30	11	14	10	3	12	17
DK	21	16	4	8	7	1	2	3	1	5
FI	42	32	13	23	6	1	4	1	3	7
FR	73	44	25	26	9	8	3	8	5	23
DE	63	31	16	33	21	7	6	17	10	21
GR	36	32	16	15	21	8	4	7	6	37
HU	39	22	13	11	9	5	2	2	2	16
IE	38	37	16	13	19	4	4	4	3	15
IT	57	50	13	27	7	4	4	4	2	4
NL	60	41	29	37	26	8	9	15	5	33
NO	30	19	13	14	7	4	4	6	7	5
PT	29	15	3	13	17	12	6	9	12	51
ES	66	64	24	27	14	9	11	13	18	33
SE	81	44	8	15	21	7	11	8	5	28
CH	55	34	22	29	17	7	6	11	5	25
UK	30	73	26	25	9	9	7	10	4	13
Total	882	640	306	397	270	115	105	160	107	383

studied population of laboratories. Half of them were accredited; in some cases, e.g. Norway and Hungary, only accredited laboratories were contacted (Table 5.6).

The needs expressed for CRMs/RMs by the laboratories contacted are very wide and were summarised in the form of a list per analytical sector, as given in Table 5.7. Only clearly identified matrices/chemical parameters are listed. From this table, it can be concluded that the environment and food/agriculture fields generated the largest variety of requests. Other comments were related to the high prices of CRMs and the need for Laboratory Reference Materials 'fit for purpose' to respond to urgent needs (small stock of defined shelf life). More instructions are deemed necessary on how to use the CRMs and better information on suppliers, types of materials etc. is also needed. It seems that many laboratories do not know how to find their way in the broad supply of RMs of very different — and often unknown — quality.

Beside the needs for RMs and CRMs listed in Table 5.7, many laboratories expressed their wish for more activities in quality assurance/quality control and for teaching on procedures to prepare Laboratory Reference Materials (in-house). They also expressed the need for a larger and better dissemination of information on interlaboratory schemes carried out in Europe (see Table 5.8). In this view, a European directory of existing structures responsible for the organisation of interlaboratory schemes would be most

TABLE 5.5

PURPOSE OF ANALYTICAL ACTIVITY OF CONTACTED POPULATION OF LABORATORIES

Country	Routine analysis related to:			Research	Quality control check of products
	Legislation	Standardisation	Monitoring/ Diagnosis		
Austria	11	5	15	16	22
Belgium	45	23	65	49	104
Czech Rep.	46	16	66	40	28
Denmark	22	3	14	12	11
Finland	38	5	18	36	22
France	57	23	49	39	45
Germany	16	16	35	76	40
Greece	19	10	27	46	51
Hungary	41	2	22	10	30
Ireland	40	8	27	26	22
Italy	43	4	39	30	14
Netherlands	69	36	47	58	53
Norway	19	7	25	11	20
Portugal	29	13	37	27	73
Spain	49	22	41	56	53
Sweden	69	17	55	31	52
Switzerland	47	20	47	40	33
United Kingdom	53	13	28	32	55
Total	713	243	657	635	728

welcome. Finally, it was considered necessary to provide CRM certificates in the different EU languages to facilitate the understanding and the use of the materials.

Other requests dealt with e.g. better information on terms and conditions of storage, needs for a market strategy, guidelines on the use of RMs/CRMs, fast and effective production of LRMs, collection of information about existing in-house RMs.

5.6.2. Needs and recommendations

The following section summarises round-table discussions held at a workshop on use and prospects for reference materials which was held in Bazas (France) in May 1998 based on the data collected in the study [41].

5.6.2.1. Needs for reference materials

Various questions and remarks arose from the lectures given at the workshop on the role of CRMs within the EU and the production/distribution by the Institute for Reference Materials and Measurements (IRMM). Firstly, the participants felt that the

TABLE 5.6

QA/QC SYSTEMS OF THE LABORATORIES

Country	Accredited/ certified	Not accredited or certified	Type of accreditation/certification system*			
			EN45000/ ISO 25	ISO 9000	GLP	Other or in house
AT	10	28	13	13	8	13
BE	48	109	50	63	20	35
CZ	51	55	60	16	0	37
DK	29	5	27	4	3	4
FI	46	21	61	12	3	2
FR	35	73	53	18	19	14
DE	26	87	34	13	27	38
GR	2	85	15	23	14	34
HU	69	1	59	6	2	6
IE	25	46	22	12	12	33
IT	12	59	32	13	41	7
NL	92	39	91	46	17	15
NO	38	3	35	2	1	1
PT	77	17	81	3	1	5
ES	28	92	47	21	26	44
SE	111	6	96	25	4	7
CH	45	49	44	10	14	30
UK	58	33	19	21	17	55
Total	802	808	839	321	229	380

*: several systems may be running in some institutions

selection and production of new CRMs should systematically follow a top-down approach, i.e. materials should be selected with respect to their possible support of EU policies and/or industrial needs, and not a bottom-up approach (selection of CRMs only based on their scientific merit without emphasis on their real impact). Discrepancies were noted between the needs shown by the IRMM as a request to e.g. Directorate General for agriculture of the European Commission (e.g. BSE, GMO) and the needs expressed by EU laboratories (see Table 5.7). Other remarks were made as a result of the SMT-inquiry, e.g. many of the CRMs requested by the laboratories are actually available either at BCR, NIST or other producers; furthermore, needs were expressed for trace organics whereas the sale of related CRMs by IRMM are very low: this feature actually illustrates a major lack of information rather than a lack of CRM availability.

The inquiry has shown that the needs for CRMs/RMs are widely recognised in all sectors. It should be noted that laboratories often expressed their wishes and needs in a very unclear and vague manner which tend to confirm that information on the use and availability of reference materials is not sufficient. In addition, many laboratories declared that they do not use CRMs because they are not aware of their utility.

An extensive list of CRMs needed by European laboratories is included in the inquiry

TABLE 5.7

NEEDS OF CRM AND RM EXPRESSED BY THE PARTICIPATING LABORATORIES

Environment	Food/foodstuff & agriculture	Health/safety	Industrial products	
Sediments - PCB, PAH - - pesticides - - chemical species - - organotins - - extractable elements - - particle sizes -	Fruits - pesticides - - sugar - - sulphite - - bromide -	Meat products - caseinate - - toxic chemical species - - anabolics - - vet. drugs - - androgen, estrogen compounds - - microbiology -	Atmospheric dust - heavy metals - - aldehyde - - PAH -	Polymers/Synthetic materials - monomers - - additives - - TE - - heavy metals - - physical prop. - - permeability -
Waste/Sludge matrices - trace metals - - chemical species - - PAH and pesticides - - PCB -	Oil products - heavy metals - - chemical species - - PAH in used oil - - tocopherols - - fatty acids -	Fish products - histamine - - fatty matters - - toxic chemical species - - sulphite - - nitrogen - - antibiotics - - vet. drug residues - - microbiology -	Adsorbents - VOC - - aldehyde -	Ores/Minerals and fuels - pure metals - - minerals - - chlorine, carbon in fuel -
Waste water - heavy metals - - major elements - - COD, BOD, nutrients - - PAH, PCB - - suspend. matter - - cyanides - - oil - - microbiology -	Soft drinks - trace elements - - colors - - preservatives - - additives - - sugars -	Soil matrices - leachable elements - - extractable elements - - TE - - chemical species - - volatile hydrocarbons - - pesticides, herbicides - - chlorophenols - - PAH and PCB - - mineral oil -	Incinerator ashes - heavy metals - - PAH - - dioxins - - sulphur -	Pure metals - trace metals in Cu and Fe alloys - - Pb and Zn alloys - - unalloyed Zn - - unalloyed Co - - Co and Ni alloys - - surface analysis - - steel alloys - - Ti and Zr alloys -

TABLE 5.7

CONTINUED

Environment	Food/foodstuff & agriculture		Health/safety	Industrial products
Fresh/Drinking water - trace metals - - chemical species - - organotins - - Cr(VI) - - cyanide - - PAH and pesticides - - nutrients, COD - - phenols, hydrocarbons - - detergents, oil - - microbiology -	Wine/alcohols - acids - - trace metals - - alcohol - - additives - - vitamins - - pesticides - - amino acids - - biogenic amines - - alcohol/water mixtures -	Compost - heavy metals - - PCB, PAH -	Human tissues and body fluids - Trace elements - - As-species in urine - - chemical species - - doping agents - - drugs in body fluids - - antibiotics in tissues - - DNA-tests - - hormones in fat - - microbiology -	Textiles - pesticides - - heavy metals - Paper paste - physical testing - - heavy metals -
Groundwater - TE and major compounds - - pesticides -	Dairy/milk products - protein and fat - - acid composit. - - nitrite, choline - - vitamins, AA - - sugar - - PCB, pesticides - - aflatoxins - - caseinate - - microbiology -	Plant matrices - trace elements - - extractable elements - - chemical species - - isotopic composition - - PCB, PAH - - pesticides - - dioxins -	Products - metals in toys - - pure pharmaceuticals -	Other products - matrices rich in noble metals - - metal impurities in auto-catalysers - - PCB in recycled paper - - metals in paints - - CRM for laser ablation -

TABLE 5.7

CONTINUED

Environment	Food/foodstuff & agriculture	Health/safety	Industrial products	
Biological matrices - heavy metals - - trace elements - - nutrients - - arsenic species - - multi-species - - PCB and pesticides - - PAH -	Flour/wheat/bread - fumonisine B - - albumin and protein - - trace elements - - vitamins - - pesticides - - dietary fibres - - additives - - amino acids -	Animal feeding stuffs - heavy metals - - extractable elements - - nutrients - - natural toxins - - crude ash, fiber - - crude protein - - fat - - moisture, starch - - microbiology -	Occupational exposure - toxic substances in body fluids - - air filters with urban particles -	Glass/Ceramic - TE - - leachable elements - - transformation temperature - - microanalysis - - colour analysis - - physical testing -
Others - size determination - - environmental toxicity -	All food products - drug residues -	Fertilizers - matrix composition - - extractable elements -	Physical properties - electromagnetic - - ultrasound -	
Calibrants - arsenic species - - other chemical species -		Calibrants - phycotoxins -	Calibrants - corticosteroids - - estrogen, androgen compounds -	Calibrants - petroleum products-

AA: amino acids TE: trace elements

TABLE 5.8

OTHER NEEDS EXPRESSED BY THE PARTICIPANTS IN THE STUDY

Needs for interlaboratory studies*	Method development and validation	Proficiency testing
ring-tests in construction sector	radon in water and air	material testing
Radioactivity measurements	enrichment procedures for pathogens	metallurgical field
Cereal analysis	testing schemes for oils and fats	PCB and toxaphen congeners
Cosmetic industry	harmonisation of test methods for polymers	cosmetic industry
Chemical products	standard methods for antibodies	
bovine herpes virus type 1	reference methods for aerosols	
Comparative testing scheme for Cs-137 and Sr-90	validation protocols for microbiological analysis	
ring-tests for forensic laboratories	standard methods for soil and air sampling	
	standard methods for waste water	

*: with teaching/learning objective

report [41]. Among them, the participants in the workshop stressed the need for research for the development of new types of CRMs matching the composition of ‘real-case’ samples. A need was also expressed for calibrants, pure substances, isotope compounds and matrix materials enriched with isotopically-labelled compounds.

5.6.2.2. Representativeness

As mentioned previously in this book, CRMs should match as much as possible the composition of ‘real samples’, e.g. inhomogeneous soil samples may be more representative than finely ground powdered material which may create more problems to laboratories than actually offer them useful tools for their analytical quality assurance (i.e. finely ground soil materials are ‘easier’ to analyse than real soil samples).

The requirement for representativeness should be considered at the stage of selection of new CRM projects. The questions to be posed should be (1) what do the end users need? and (2) is it possible to produce the needed CRMs without disturbing their representativeness too much? The participants recommended that a close consultation be systematically foreseen between end-users, researchers and producers in order to define the ‘best compromise’ for representative materials with an acceptable homogeneity and stability, i.e. small batches of ‘wet’ RMs or CRMs with a limited (well-defined)

stability responding to needs may be better than large stocks of unrepresentative (and not used) CRMs. Materials have to be 'fit for purpose'; an example is the development of microbiological CRMs with certified values guaranteed at $\pm 100\%$, which correspond to the present state-of-the-art and clearly fulfil a strong demand.

5.6.2.3. Development and CRM production

It was assumed that the production of most obvious CRMs (i.e. 'simple' matrix CRMs for classical parameters that were already produced in the past) could be tackled by commercial companies. The role of the European Commission is felt to be necessary when the technological risks for CRM production are significant. The development of CRMs is not a 'normal' market since it involves very specialised manufacturing procedures. No commercial company can afford to develop CRMs in support of many purposes. Quality is the key word: commercial materials might not be of sufficient quality to respond to the demand. The EC should act where national initiatives cannot comply with the demand. Participants considered that reference materials should be certified at the EC level, not at the national level, and that commercial products should refer to 'primary' (certified) materials. A scheme should be developed for the mutual acceptance of materials, e.g. establishing a kind of 'EC label of quality'; in this context, accreditation of RM producers should become mandatory.

It was generally agreed that the development of CRMs should be linked to practical needs, i.e. in support of legislation, industry and trade issues, and monitoring. For legislation purposes, requests should originate from the EC General Directorates, i.e. Directives should systematically include quality requirements, including the use of CRMs. This is already the case in many instances for directives related to food and new framework directives (e.g. the new framework directive on drinking water) but this should be extended to other areas. Participants recommended that systematic interservice consultations within the EC be carried out to define the main areas of interest for QA matters.

5.6.2.4. Interlaboratory trials

Intercomparisons to improve or monitor the performance of analytical methods are often preferred over the use of CRMs. The collaborative (learning) aspects are felt to be very valuable by EC laboratories. The Commission should continue to consider, on a case-by-case basis (following well-defined selection criteria), various types of interlaboratory trials such as e.g. improvement schemes (stepwise learning programme to improve the state-of-the-art of a field of analysis), method performance studies (to improve a specific method, e.g. a standardised method), interlaboratory studies involving various types of methods (network of laboratories prior to a certification), and proficiency testing schemes (involving 'real-case' materials prepared at regular intervals).

For trade issues, proficiency testing could also respond to the need for improving measurement quality. The EC should stimulate the establishment of links between various proficiency testing schemes in order to make the best use of them.

5.6.2.5. *Information/Education/Training*

The lack of knowledge and information is one of the most critical issues which hampers the wider use of reference materials. General information is scarcely available and in most cases it is in the English language; technicians and many laboratory managers will only read information if available in their own language. Training activities are very few and almost non-existent at the university level. It is one thing to publish information, another to put it into practice. Information published in the scientific literature or books is generally poorly followed, which is again partly due to the fact that most of the published papers are in English.

The problem of 'too high' costs of CRMs was also debated. It was stressed that nobody complains about the high costs of e.g. suprapure acids, which are mandatory for trace analysis. The perception of high costs hence seems to be related to a problem of 'culture' and education. Unless the education background is established, the awareness will remain at a very low level and the market will not develop. Information should appear in all academic training; teachers need also to be trained!

Many laboratories do not use reference materials because they are not forced to do so which imply that the users of the data should primarily be briefed on the importance of CRMs as a tool for the quality of data.

Participants felt that the EC should act as a catalyst for establishing structures for the dissemination of information/knowledge on the best practice for use of CRMs/RMs and information on available materials. There is an obvious need for a wide marketing of existing products in order to boost the use of CRMs, which should be conducted by the EC. In addition, protocols for preparing 'in-house' reference materials, including instructions for transport, storage etc. would also be a valuable step forward. These are related to the needs of education to establish common background of understanding.

5.6.2.6. *Networking institutes/Databases*

A systematic collection of information from different research groups developing in-house RMs for their own needs would be of considerable value for many analytical sectors. This collection could be achieved by networking institutes. Participants stressed that a lot of information is available on attempts to certify CRMs, which could be of considerable help for the preparation of LRMs.

Networks may enable the exchange of expertise (including exchange of technicians), materials or activities in order to achieve complementarity within one sector of activity or within several sectors. Participants strongly recommended that such types of networks be established in the area of CRM/RM production and use (networking research institutes, producers and end-users). While the production of the vast majority of CRMs can be tackled through 'classical' projects, other needs could be fulfilled by developing structures in the form of networks, e.g. materials urgently needed, materials for proficiency testing schemes, materials required for research etc.

Such types of structure could also offer facilities for laboratories to test their procedures on-site in the case where CRMs cannot be produced, e.g. 'reference sites'

for testing sampling methodologies (e.g. for waste, soil etc.), or comparing methods on the same sample (e.g. polymer paste, waste water etc.).

Beside the network needs, there is a clear need to establish databases in Europe of all existing RMs which were developed not only in recognised production centres (as mentioned in section 5.6, such databases are already available, e.g. the COMAR databases) but also in small production units and laboratories. This collection and exchange of information could also be carried out through networking and a forum for 'questions-responses' could be developed e.g. through Internet; this should include possibilities for seeking needs, collect data etc. under the umbrella of the EC.

5.6.3. Summary of needs

New production strategies for CRMs will have to be global [42]. The real work for setting strategies rests with the future producers of reference materials [43]. A first strategy for RM producers could centre on developing systematic collection and monitoring of trends that affect the field. The Reference Materials Committee (REMCO) of the International Organisation for Standardisation (ISO) has made some limited contributions along these lines. Surveys have been developed and distributed among producers [26]. A second strategy could focus on developing greater cooperation among producers which could be facilitated by the World Wide Web [43]; to date, formal agreements to promote cooperation have been limited in success, partly by overly bureaucratic structures. The WWW does not suffer from formal bureaucracy and CRM producers could make their instruments and laboratories just as open and available to researchers who are characterising new materials. Thirdly, increased attention could be focused on accreditation of RM producers; an emphasis on increasing quality and confidence would indeed make the growth rate more productive in meeting RM needs, while at the same time saving resources. A fourth strategy for RM producers centres on obtaining greater support for research, production and certification of RMs. Increasingly, laboratories must convince policy level managers that RMs serve major objectives of human welfare.

Accreditation is mainly concerned with routine tests or analysis. It includes the use of reference materials (e.g. LRMs or in-house RMs) for control charts which are hardly available on the market; it is hence timely to start producing RMs for routine analysis in high quantities and at low cost for those measurements which have a major economic importance in relation to social and environmental implications; as mentioned above, such a production should follow minimum quality requirements to avoid commercialisation and use of bad quality products which would only add to confusion. There is obviously a need to focus/consolidate technical expertise for CRM production in a few centres of excellence and actions should be stimulated to create a possible network of CRM producers which should be composed of 'practitioners', i.e. experts in both the production of CRMs and the respective analytical fields, so that questions arising from routine laboratories could be readily and practically responded to [44]; while efforts made by metrological laboratories to create a system of 'metrology in chemistry' are certainly important, these are far remote from the needs of routine laboratories which, however, carry out the vast majority of analytical work worldwide. Pragmatism should

be directed towards help given to end users (analysts) through a network composed of CRM producers with analytical expertise and not only theoreticians.

The systematic verification of the performance of methods should also be strengthened. In some cases this is not possible owing to a lack of calibrants of suitable stoichiometry and purity (e.g. in the field of speciation analysis). The production of new calibrants should hence be envisaged, particularly as it was shown that, in environmental analyses, pure substances or calibration solutions obtained from various suppliers might differ significantly [45].

Matrix-matched CRMs are increasingly needed for a range of environmental matrices, elements or compounds, including materials certified for operationally-defined parameters (e.g. extractable trace element contents). Finally, training on the preparation of LRMs and use of CRMs should be systematised in the framework of accreditation systems. Such courses already exist [46] but are not fitted for purpose with respect to accreditation; they should involve advice on how to develop LRMs versus CRMs, use RMs in sectorial proficiency testing (e.g. environmental monitoring) etc.

5.6.4. Expected trends

The growing raft of environmental regulations and quality requirements has driven the demand for reference materials at more than 20% per year [42]. Clearly, existing producers cannot meet the future demand owing to budget cuts in many government programmes. It should be noted that the cost of production of reference materials, especially matrix CRMs, is very high, and the user pays only a small part of the real price [47]. This means that true commercial production is very unlikely, so new methods of production must be found. One solution may be to reduce demand for CRMs by making available secondary RMs, designed for day-to-day use. Such a development will depend on specialist producers who have the perceived quality systems and ability to make a product that is traceable to a CRM, and the ability to support the customer who uses the product. Suitable organisations are few and should demonstrate the necessary quality standard through ISO 9000 certification of procedures that specify absolute adherence to the appropriate ISO guides concerning the production and use of reference materials; they should also have an efficient international sales and customer support service [47].

A semi-quantitative estimation was recently proposed by NIST [43] along the following orientations :

Ten-year horizon, considering the recent evolution in the development of measurement instruments, the CRM demand should grow about 5% per annum; because their usage is growing from a smaller base, the needs for non-certified reference materials (e.g. for proficiency testing in support of laboratory accreditation) should increase by 10% per annum.

Twenty-year horizon, the use of CRMs should continue to grow at a rate of 5% per annum. The needs in terms of the quality of routine measurements should yield to an increasing use of non-certified reference materials (linked to accreditation) at a rate of 15% per annum.

Thirty-year horizon, the increasing sophistication of measurement instruments should enable the resolving of all difficulties inherent to validation and the use of CRMs should hence decrease; it is likely that the needs will be directed towards the continued replacement of obsolete materials with new types of materials to address the challenges. Needs for materials linked to accreditation will likely slow to a still high rate of 5% per annum.

5.6.5. Conclusions

The inquiry report concludes that the utility and use of reference materials are insufficiently known. Too many materials are considered as 'certified', which shows that many laboratories do not exactly know what is meant by a CRM. Therefore, the most urgent action to be undertaken is certainly information: this is illustrated by the number of requests shown in Table 5.7 for which many CRMs actually exist! Laboratories should be informed about the type(s) of materials they should use, and how they should use them; this may vary considerably from one field of analysis to another, and tailor-made recommendations would be preferred over general rules. An overview of existing reference materials would be of great help. The same applies to interlaboratory studies for which the number is very high and which are often restricted to narrow fields; information on how to use results of these schemes should be disseminated to avoid laboratories erroneously using consensus values as 'true values'. Finally, a marketing survey for reference materials should be performed. A much more detailed level of information could be collected if the study would aim at laboratories operating in a specialised field only. Much remains to be done to establish a firm infrastructure, which will require close cooperation among laboratories, research institutes and reference material producers.

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Annex 5.1

Determination of Pentachlorophenol (PCP) and five selected Polycyclic Aromatic Hydrocarbons (PAH) in wood — Certification Study

EC-Project: SMT4-CT97-2147

Spring 1999

PROTOCOL FOR REPORTING RESULTS

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*Annex 5.1 (continued)***1. General**

The following information is given to ensure that all participants will be able to supply the necessary information and data in a common format and to enable the confirmation and comparison of the traceability and quality of the data.

The analytes to be determined in the candidate CRM are Pentachlorophenol (PCP) and the five selected Polycyclic Aromatic Hydrocarbons (PAH):

- Benzo[*a*]anthracene
- Benzo[*a*]pyrene
- Benzo[*e*]pyrene
- Benzo[*b*]fluoranthene
- Benzo[*k*]fluoranthene.

Completion of the following tasks and the corresponding reporting forms is a minimum pre-requisite for the fulfilment of the certification exercise.

2. Warning

The wood samples supplied contain high levels of PAH, that may pose health risks for personnel when treated inappropriately and may contaminate lab workplace environment.

3. Materials provided

- three bottles containing approx. 60 g of a contaminated wood sample, the candidate CRM
- two bottles containing approx. 36 g of a blank wood sample
- two CERTAN capillary bottles with a solution of PCP and the five PAH in toluene with known concentration levels ('known solution')
- two CERTAN capillary bottles with a solution of PCP and the five PAH in toluene with approximately given nominal concentration levels ('unknown solution')¹

4. Analytical work**4.1. Calibration**

All analytical work is to be done with own calibrant solutions prepared — according to the BCR regulations — from pure CRM or (if not possible) from the best verified

¹This so-called 'unknown solution' is prepared in exactly the same way as the 'known solution', which implies that the exact concentration is known from preparation and verification. These data will be used in evaluating the results of the certification study.

Annex 5.1 (continued)

product, preferably certified, as available and under precautions to guarantee a maximum of traceability (see BCR Guide, provided for the Feasibility study). Prepare at least a 4 point's calibration curve for each compound to be determined. Prepare, if appropriate, internal standard/calibration solutions by mixing suitable mass or volume aliquots of calibrant and internal standard solution. Please use at least one internal standard for PAH analysis and one for PCP analysis.

4.2. Determination of the concentration of the provided 'known solution'

Countercheck the given concentrations of the provided known synthetic solution versus the values obtained from your own calibrants. In case there is a serious deviation from the concentrations as specified, please consult the co-ordinator before proceeding.²

Please note that this solution as well as the 'unknown solution' was prepared in all steps only by gravimetry. Therefore all concentrations in the reporting sheets are given in mg/kg. If you use volumetric techniques for the analyses of these solutions please divide your results in mg/L by the density of Toluene as given here for two different standard temperatures as used for temperature control in volumetry:

$$D_{4}^{20} = 0,8661 \text{ kg/L} \quad D_{4}^{25} = 0,8616 \text{ kg/L}$$

The uncertainty of these values is less than $\pm 0,0003 \text{ kg/L}$ as found by literature and as checked by own experiments.

4.3. Determination of the reagent blank

Perform your analytical procedure without the components. Calculate the results as if the same test portion as in your sample determination were taken for analysis. Please correct the results for the sample if appropriate for the blank. ***Please, remember that very high reagent blanks are revealing the use of inappropriate chemicals, glassware or methods. Action should be taken in case such blanks occur to find the contamination source***³.

4.4. Determination of the concentration of the provided 'unknown solution'

The provided 'unknown solution' contains the six analytes in a level of concentration not higher than that of the provided known solution. The determination of the analytes

²The deviations of measurement results from the gravimetry based values of analyte contents of the 'known solution' given in sheet 2 of the reporting sheets should be within the level of approximately $\pm 5 \%$ as given there in the field 'Uncertainty' for each analyte as a limit for acceptable deviations from the gravimetric values. The uncertainties of the certified purities of the CRM used for the preparation of the solutions are included in this uncertainty figure based on our experience with different methods used for verification. It is not the uncertainty figure of the gravimetric procedure which is much lower.

³All comments in this paper stressed by bold italic letters were made by the European Commission. As they point out some very important aspects according to the BCR regulations concerning the certification study they should be especially taken into consideration by all participants.

Annex 5.1 (continued)

in the provided 'unknown solution' is to be made in accordance with the normal procedure of your laboratory. Please make six separate determinations of the analytes in *this 'unknown solution'*, at least at two days, preferably *in* parallel to the measurement of the contaminated wood samples.

4.5. Determination of the blank wood sample

Make at least two separate determinations of the analytes in the blank wood sample, i.e. separate sample extractions. Please report — under all conditions — a numerical value. Values reported as ('< x'), where x denotes a detection and/or determination limit, **are not informative and therefore not acceptable**. You can state the detection limit in your method description. Comments as 'not detectable' are also not accepted.

4.6. Determination of the contaminated wood sample

Make six separate determinations of the analytes — i.e. separate sample extractions — **from two separate bottles of** contaminated wood at different (at least at two) days with a refreshed calibrant solution each day. For every extraction make at least one measurement. Please report all results for all analytes.

4.7. Determination of the water content of the contaminated wood sample

At least three samples **from each of the two used bottles** of the contaminated wood sample provided should be analysed for water content by heating them at 102 ... 105°C until constant weight. These **test** samples should not be used for PAH and PCP determination. The determination of the water content must be performed on the same day as the analyte determination and from the same bottle as used for this purpose.

4.8. Recovery measurements

Make four recovery measurements by spiking the contaminated wood sample with a known amount of the analytes. The spiking level shall be varied in each recovery experiment from 50% to 200% of the target value of the contaminated wood sample. The material has to be allowed to absorb the spiked analyte at least 24 hours.

Two laboratories were allowed to deviate from this procedure (laboratory numbers refer to the feasibility study)

- (1) Lab. 9, using the SFE method may use the blank wood sample for spiking
- (2) Lab. 6, using a procedure without derivatisation or clean up may use the method of multiple extraction but has to use at least two different solvents tested before to be appropriate.

*Annex 5.1 (continued)***4.9. Further instructions**

The minimum sample intake for the determination of the contents of the six analytes in the contaminated wood sample as well in the blank wood sample and also for the recovery experiments is 1,0 g of wood.

Each laboratory should work under *reproducible* conditions. All necessary measurements should be performed by one operator using only one instrument. If there is clearly a best instrument or a best operator, use these only.

- Analyte concentrations shall be related to mass, i. e. as mg / kg
- At least one internal standard must be used.
- The purity of internal standard(s) and calibrant(s) and its verification must be reported.

5. Reporting of Results

Please:

- use the Excel-spreadsheet as provided
- do not change in any way the structure or layout of the file
- express the results to at least 3 significant figures
- give all details requested of the method in a ‘telegram-style’
- submit a description of the detailed operational procedure and
- supply additional information as a Word-document, if appropriate.

Provide representative copies of⁴:

- calibration plot(s)
- one chromatogram of the known solution and one of the standard solution
- one chromatogram of the reagent blank and one of the wood blank
- one chromatogram of each contaminated wood sample

6. Return of Results

Please return results by E-mail to

⁴Please bring these documents at the meeting only. If they are needed for evaluation purposes, you will kindly be asked to forward them if deemed necessary by the co-ordinator/statistician. In principle, most of the context needed for the statistician is provided in the Excel-reporting sheets and the description of the measurement procedure.

Certification Study within the framework of project SMT4-CT97-2147

E-mail

End of measurements (date)

[illegible][illegible]

Composition	Value	Uncertainty
Pentachlorophenol	0.492	0.022
Benzo[a]anthracene	0.479	0.020
Benzo[a]pyrene	0.459	0.023
Benzo[e]pyrene	0.561	0.030
Benzo[b]fluoranthene	0.676	0.032
Benzo[k]fluoranthene	0.397	0.019

All data are to be entered/have been entered in mg/kg
Uncertainties are to be interpreted as confidence intervals

II. Results on the "unknown solution" (mg/kg)

	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Date of analysis									
Sample identity									
Pentachlorophenol									0

	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Date of analysis									
Sample identity									
Benzo[a]anthracene									0
Benzo[a]pyrene									0
Benzo[e]pyrene									0
Benzo[b]fluoranthene									0
Benzo[k]fluoranthene									0

Nominal composition	Value
Pentachlorophenol	0.4
Benzo[a]anthracene	0.3
Benzo[a]pyrene	0.4
Benzo[e]pyrene	0.4
Benzo[b]fluoranthene	0.5
Benzo[k]fluoranthene	0.3

All data are to be entered/have been entered in mg/kg

III. Results on the contaminated wood sample (mg/kg)

Raw Data (A)	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Date of analysis									
Sample identity									
Pentachlorophenol									0

Raw Data (A)	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Date of analysis									
Sample identity									
Benzo[a]anthracene									0
Benzo[a]pyrene									0
Benzo[e]pyrene									0
Benzo[b]fluoranthene									0
Benzo[k]fluoranthene									0

All data are to be entered/have been entered in mg/kg

Determination of Moisture content

Raw Data (A)	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Date of analysis									
Sample identity									
Moisture content (%)									0

NOTE: For each value of table (A), a value for moisture is to be given; for values in table obtained at the same day, the same moisture content may be used

Data on basis of dry matter(B)	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Pentachlorophenol	0	0	0	0	0	0	0	0	6
Benzo[a]anthracene	0	0	0	0	0	0	0	0	6
Benzo[a]pyrene	0	0	0	0	0	0	0	0	6
Benzo[e]pyrene	0	0	0	0	0	0	0	0	6
Benzo[b]fluoranthene	0	0	0	0	0	0	0	0	6
Benzo[k]fluoranthene	0	0	0	0	0	0	0	0	6

NOTE : Table B is calculated automatically

Final result (C)	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Pentachlorophenol									0
Benzo[a]anthracene									0
Benzo[a]pyrene									0
Benzo[e]pyrene									0
Benzo[b]fluoranthene									0
Benzo[k]fluoranthene									0

NOTE : The final result (C) equals the result on dry basis (B), corrected for recovery.

NOTE : Table C is calculated automatically

IV. Results on the blank wood sample (mg/kg)

Raw Data (A)	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Date of analysis									
Sample identity									
Pentachlorophenol									0

Raw Data (A)	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	Replicate #6	Average	Stddev	Count
Date of analysis									
Sample identity									
Benzo[a]anthracene									0
Benzo[a]pyrene									0
Benzo[e]pyrene									0
Benzo[b]fluoranthene									0
Benzo[k]fluoranthene									0

All data are to be entered/have been entered in mg/kg

Annex 5.2 (continued)

Method descriptions:**I. "Known solution"****Pentachlorophenol****Benzo[a]anthracene****Benzo[a]pyrene****Benzo[e]pyrene****Benzo[b]fluoranthene****Benzo[k]fluoranthene**

Aliquote for analysis (mL)

Solvent exchange (yes/no)

Alkaline or acid pretreatment

Derivatisation

Clean up

Dilution solvent(s)

Dilution factor

Injection volume (mL)

Separation technique

Detection technique

Detection limit

Internal Standard I

when added

Purity of substance

Uncertainty of purity

Internal Standard II

when added

Purity of substance

Uncertainty of purity

Annex 5.2 (continued)

II. "Unknown solution"	Pentachlorophenol	Benzo[a]anthracene	Benzo[a]pyrene	Benzo[e]pyrene	Benzo[b]fluoranthene	Benzo[k]fluoranthene
Aliquote for analysis (mL)						
Solvent exchange						
Alkaline or acid pretreatment						
Derivatisation						
Dilution solvent(s)						
Dilution factor						
Injection volume (mL)						
Separation technique						
Detection technique						
Detection limit						
Internal Standard I when added						
Purity of substance						
Uncertainty of purity						
Internal Standard II when added						
Purity of substance						
Uncertainty of purity						

Annex 5.2 (continued)

III. Wood	Pentachlorophenol	Benzo[a]anthracene	Benzo[a]pyrene	Benzo[e]pyrene	Benzo[b]fluoranthene	Benzo[k]fluoranthene
Extraction technique						
Extraction solvent(s)						
Sample amount (g)						
Final volume (mL)						
Aliquote for analysis (mL)						
Solvent exchange						
Alkaline or acid pretreatment						
Derivatisation						
Clean up						
Dilution solvent(s)						
Dilution factor						
Injection volume (mL)						
Separation technique						
Detection technique						
Internal Standard I when added						
Purity of substance						
Uncertainty of purity						
Internal Standard II when added						
Purity of substance						
Uncertainty of purity						

V. Recovery experiment for Benzo [a] anthracene

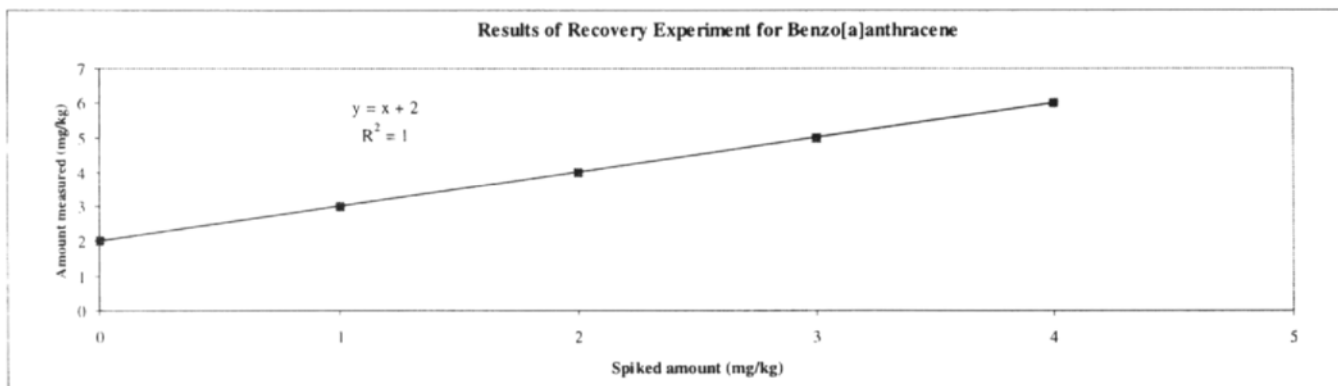
Recovery level	Amount Spiked (μg of analyte/ g of wood)	Amount measured (μg of analyte/g of wood)
nothing added	0	2
50 % added	1	3
100% added	2	4
150% added	3	5
200% added	4	6

Regression analysis

Model: $y = a + bx$

Slope (b):	1.0000	2.0000	Intercept (a)
Error of the slope:	0.0000	0.0000	Error of the intercept
r^2	1.0000	0.0000	s of residual

Recovery (%)	100.0
standard error:	0.0



Note: this sheet will be available for every parameter to be determined

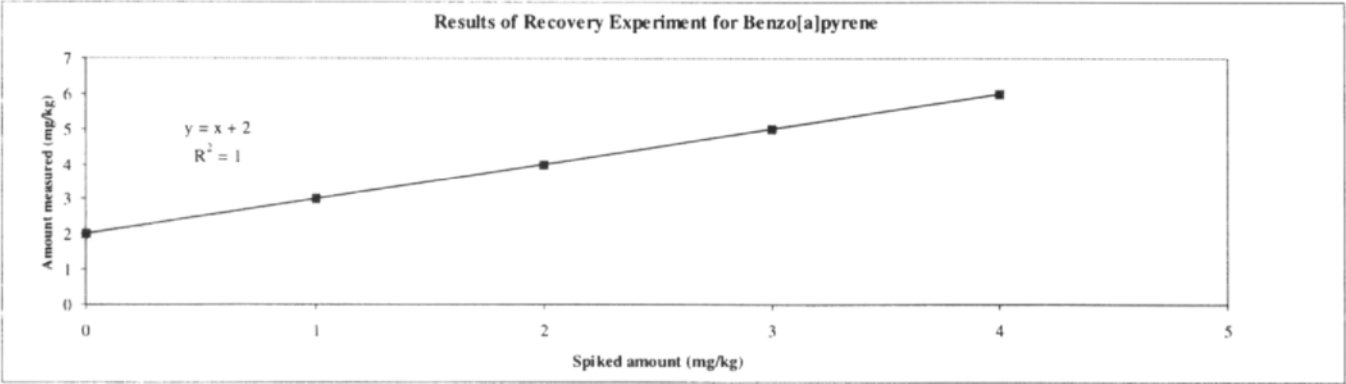
V. Recovery experiment for Benzo[a]pyrene

Recovery level	Amount Spiked (µg of analyte/ g of wood)	Amount measured (µg of analyte/g of wood)
nothing added	0	2
50 % added	1	3
100% added	2	4
150% added	3	5
200% added	4	6

Regression analysis
Model: $y = a + bx$

Slope (b):	1.0000	2.0000	Intercept (a)
Error of the slope:	0.0000	0.0000	Error of the intercept
r^2	1.0000	0.0000	s of residual

Recovery (%)	100.0
standard error:	0.0



Note: this sheet will be available for every parameter to be determined

V. Recovery experiment for Benzo [e] pyrene

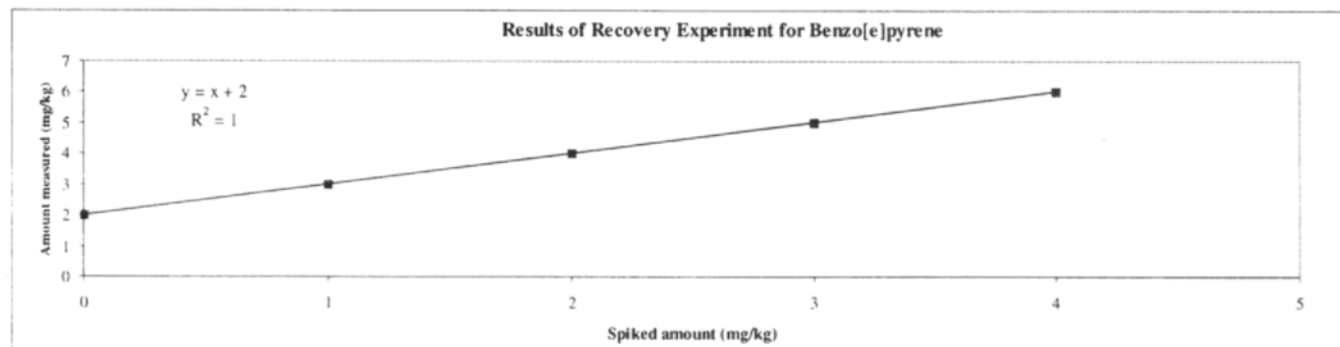
Recovery level	Amount Spiked (μg of analyte/ g of wood)	Amount measured (μg of analyte/g of wood)
nothing added	0	2
50 % added	1	3
100% added	2	4
150% added	3	5
200% added	4	6

Regression analysis

Model: $y = a + bx$

Slope (b):	1.0000	2.0000	Intercept (a)
Error of the slope:	0.0000	0.0000	Error of the intercept
r^2	1.0000	0.0000	s of residual

Recovery (%)	100.0
standard error:	0.0



Note: this sheet will be available for every parameter to be determined

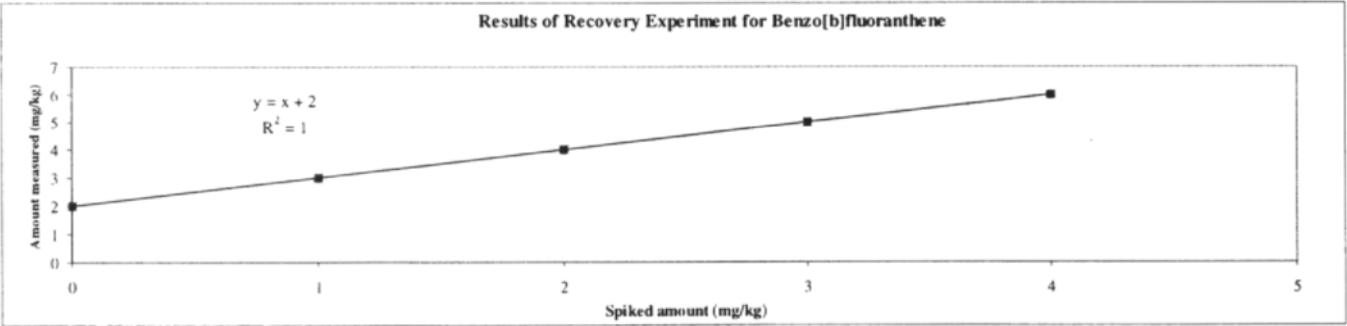
V. Recovery experiment for Benzo [b] fluoranthene

Recovery level	Amount Spiked (µg of analyte/ g of wood)	Amount Measured (µg of analyte/g of wood)
nothing added	0	2
50 % added	1	3
100% added	2	4
150% added	3	5
200% added	4	6

Regression analysis
Model: $y = a + bx$

Slope (b):	1.0000	2.0000	Intercept (a)
Error of the slope:	0.0000	0.0000	Error of the intercept
r^2	1.0000	0.0000	s of residual

Recovery (%)	100.0
standard error:	0.0



Note: this sheet will be available for every parameter to be determined

V. Recovery experiment for Benzo [k] fluoranthene

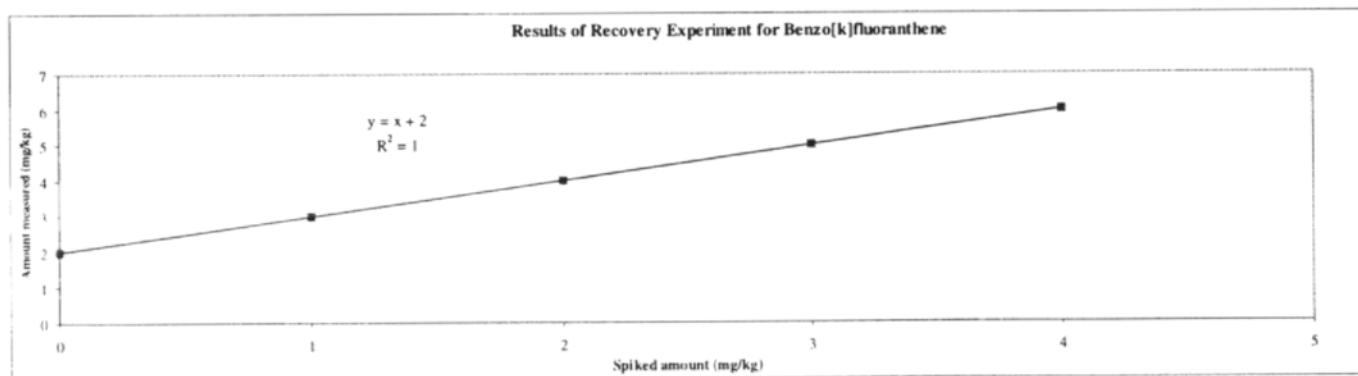
Recovery level	Amount Spiked (μg of analyte/ g of wood)	Amount Measured (μg of analyte/g of wood)
nothing added	0	2
50 % added	1	3
100% added	2	4
150% added	3	5
200% added	4	6

Regression analysis

Model: $y = a + bx$

Slope (b):	1.0000	2.0000	Intercept (a)
Error of the slope:	0.0000	0.0000	Error of the intercept
r^2	1.0000	0.0000	s of residual

Recovery (%)	100.0
standard error :	0.0



Note: this sheet will be available for every parameter to be determined

V. Recovery experiment for Pentachlorophenol

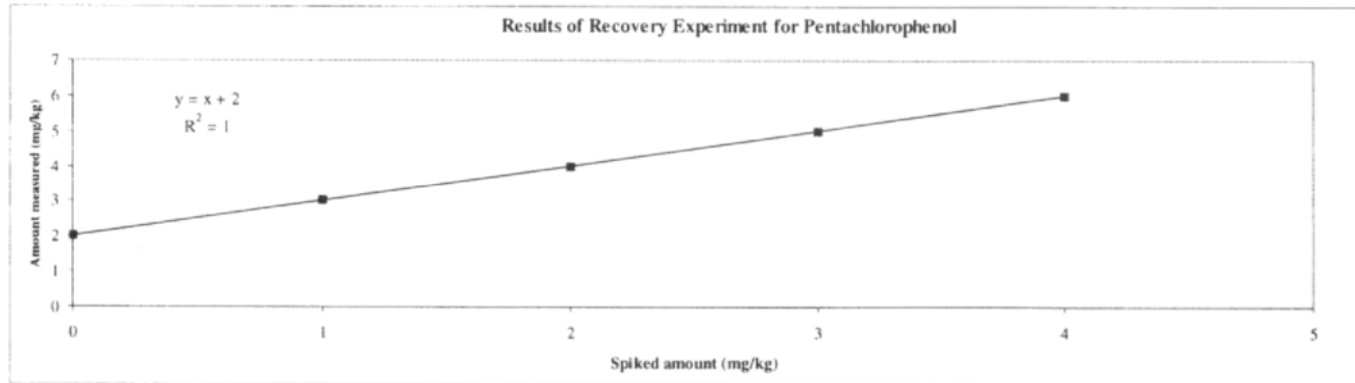
Recovery level	Amount Spiked (μg of analyte/ g of wood)	Amount Measured (μg of analyte/g of wood)
nothing added	0	2
50 % added	1	3
100% added	2	4
150% added	3	5
200% added	4	6

Regression analysis

Model: $y = a + bx$

Slope (b):	1.0000	2.0000	Intercept (a)
Error of the slope:	0.0000	0.0000	Error of the intercept
r^2	1.0000	0.0000	s of residual

Recovery (%)	100.0
standard error :	0.0



Note: this sheet will be available for every parameter to be determined

Annex 5.3

COMMISSION OF THE EUROPEAN COMMUNITIES

COMMUNITY BUREAU OF REFERENCE — BCR

CERTIFIED REFERENCE MATERIAL

CERTIFICATE OF ANALYSIS

CRM 529

CHLOROPHENOLS, CHLOROBENZENES, POLYCHLORODIBENZO-p-DIOXINS
AND POLYCHLORODIBENZO FURANS IN INDUSTRIAL SOIL
(sandy soil)

Compound	Mass fraction		Number of accepted sets of results p
	Certified value expressed as (1)	Uncertainty expressed as (2)	
	mg.kg ⁻¹	mg.kg ⁻¹	
1,2,3-trichlorobenzene	0.63	0.11	7
1,2,3,4-tetrachlorobenzene	1.6	0.3	10
Pentachlorobenzene	1.3	0.3	10
3,4-dichlorophenol	0.23	0.04	7
2,4,5-trichlorophenol	1.51	0.10	7
Pentachlorophenol	0.23	0.04	8
	µg.kg ⁻¹	µg.kg ⁻¹	
2,3,7,8-TCDD	4.5	0.6	12
1,2,3,7,8-PeCDD	0.44	0.05	6
1,2,3,4,7,8-HxCDD	1.2	0.3	9
1,2,3,6,7,8-HxCDD	5.4	0.9	11
1,2,3,7,8,9-HxCDD	3.0	0.4	12
2,3,7,8-TCDF	0.78	0.13	7
1,2,3,7,8-PeCDF	0.14	0.03	8
2,3,4,7,8-PeCDF	0.36	0.07	8
1,2,3,4,7,8-HxCDF	3.4	0.5	9
1,2,3,6,7,8-HxCDF	1.09	0.15	12
1,2,3,7,8,9-HxCDF	0.022	0.010	5
2,3,4,6,7,8-HxCDF	0.37	0.04	12

(1) This value is the unweighed mean of the means of p accepted sets of results.

(2) The uncertainty is taken as the half-width of the 95% confidence interval of the mean given in (1). When the reference material is used to assess the performance of a method, the user should refer to the recommendations of the certification report.

*Annex 5.3 (continued)***INSTRUCTIONS FOR USE**

For analysis the sample should be taken as it is. The water content is approximately 1.5% by mass. The samples should be stored at room temperature. The recommended sample intake is 2 g.

WARNING

Toxic material. The material must be handled with great care, especially avoiding skin contamination, ingestion or inhalation.

Brussels, November 1998

BCR for certified true copy

DESCRIPTION OF THE SAMPLE

The sample consists of approximately 50 g of industrial sandy soil in brown glass bottles with a polythene insert lined with aluminium. Additional information on the presence of additional organic chlorinated compounds other than those tabulated is given in the report.

PARTICIPATING LABORATORIES**2.1 Preparation, Homogeneity and Stability Studies**

- Institut Fresenius, Ingelheim, Germany
- Institut Fresenius, Hamburg, Germany
- Institut Fresenius, Dresden, Germany
- Institut Fresenius, Dortmund, Germany
- Institute for Reference Materials and Measurements, Joint Research Centre, European Commission, Geel, (Belgium).

2.2 Certification analysis of chlorobenzenes and chlorophenols

- Bayer AG, ZF-DZA, Leverkusen, Germany
- Institut Fresenius, Dortmund, Germany
- Institut Pasteur de Lille, Departement Eau et Environnement, Lille, France
- IRH, Génie de l'Environnement, Vandoeuvre, France
- Miljoe-Kemie, Albertslund, Denmark
- National Public Health Institute, Division of Environmental Health, Kuopio, Finland
- RIVM, Laboratory of Organic Analytical Chemistry, Bilthoven, The Netherlands
- RW-TÜV, Anlagetechnik GmbH, Essen, Germany
- Universidad de Santiago de Compostela, Dpto. Quimica Analitica, Santiago, Spain
- Universitat de Barcelona,, Dpt. Quimica Analitica
- Universiteit van Amsterdam, MTC, Amsterdam, The Netherlands
- VTT Chemical Technology, Chemical Analysis, Espoo, Finland

*Annex 5.3 (continued)***2.3 Certification analysis of the PCDD and PCDF**

- Centre d'Analyse et de Recherche sur les Substances Organiques — CARSO, Vernaison, France
- Centro de Investigacion y Desarrollo, CID-CSIC, Barcelona, Spain
- CSL Food Science Laboratory, Norwich, Great Britain
- CNR, Istituto Inquinamento Atmosferico, Roma, Italy
- EMPA, Dübendorf, Switzerland
- Institut Fresenius, Ingelheim, Germany
- Institute of Occupational Health, Dept. of Occ. Hygiene and Toxicology, Helsinki, Finland
- Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy
- National Public Health Institute, Division of Environmental Health, Kuopio, Finland
- Solvay Duphar B.V., Environmental Research Dept., Weesp, The Netherlands
- University of Umea, Dept. of Environmental Chemistry, Umea, Sweden
- VITO, Vlaamse Instelling voor Technologisch Onderzoek, Mol (Belgium)
- VTT Chemical Technology, Chemical Analysis, Espoo (Finland)
- ZENECA Specialities, Manchester, Great Britain

METHOD USED

Calibration was done with BCR-RM's (PCDD/F) or compounds of verified purity and stoichiometry (CB and CP). The samples were extracted with one or a mixture of solvents and were cleaned-up before or after derivatisation (CP only). Separation and quantification was carried out by HPLC with amperometric detection (CP) or gas chromatography with ECD, MS or atomic emission detection.

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NOTE

A detailed technical report on the analytical procedure and the treatment of the analytical data is supplied with each sample.

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Chapter 6

CRMs for plant analysis

6.1. TRACE ELEMENTS IN AQUATIC PLANTS

6.1.1. Introduction

Aquatic plants are used in environmental monitoring to identify point source pollution and study contamination trends. The materials presented in this section were selected as being representative of aquatic plants usually used in environmental monitoring (CRMs 060 and 061). In addition, a material of olive leaves was also certified for the quality control of determinations of trace elements of nutritive importance (CRM 062). The three materials were certified for their contents of Cd, Hg, Mg, Pb and Zn in 1990; an additional certification of Al was carried out in 1987. They were selected from a group of eighteen materials prepared by the Joint Research Centre of Ispra (Italy) [1]. The selection criteria focused on the element contents, from low levels for background studies to high levels for environmental monitoring or geobotanical prospecting, and on the analytical difficulties likely to be expected from the plant matrices.

Submerged aquatic plants show the required increase (with respect to terrestrial plants) element concentrations. *Lagarosiphon major* (CRM 060) is a typical species found in nearly all European fresh water bodies. Even higher element concentrations are present in aquatic mosses, like CRM 061 (*Platihypnidium riparioides*). These mosses combine the specific heavy metal accumulation ability of the bryophytes (leading to their use in environmental monitoring) with the general tendency of aquatic plants to collect actively metals from the surrounding water (due to their special nutritional functions). Fat in plants causes difficulties in achieving a complete digestion. Therefore, olive leaves, containing fat, are interesting for the analytical chemist. The fat content of material CRM 062, however, is still low enough to allow the formation of a dry powder without lumping. An increased interest for the environmental monitoring of Al (toxic to fish and human) has justified an additional certification of this element in the three plant materials.

6.1.2. Production of the materials

In the preparation of each of the three materials about one ton of fresh material was collected, washed carefully and air-dried. Drying was completed at 100°C and then the material was exposed to a temperature of 130°C for several hours. The resulting material was ground by ball-milling and passed through a 125 µm sieve. The fraction with a particle size smaller than 125 µm was collected in a plastic homogenising drum of 180 L

and homogenised. A preliminary check of the homogeneity was made for the elements Fe, Mn, K, P, Ca and Zn by X-ray fluorescence on pellets of 2 g.

Aliquots of about 25 g were transferred into well-cleaned glass bottles of 100 mL and sealed under a plastic insert and a screw cap. After filling 40 bottles, the homogenisation drum was closed again and the remaining material was mixed again during about ten minutes; then the filling of another 40 bottles was carried out.

For the preparation of the CRM 060, a submerged aquatic species which occurs in Lago Maggiore (Italy) was treated by the general method described above. A slightly different procedure was necessary for the CRM 061, a submerged aquatic moss collected from a tributary of the same lake; in this case it was necessary to manually remove small limestone particles attached to the moss fibres; a tungsten carbide hammer mill was used for grinding.

The CRM 062 consisted of freshly picked olive twigs from Pescara (Italy). After washing, the twigs were air-dried on a plastic lattice positioned ca. 40 cm above a layer of polythene. The leaves were removed by threshing with wooden rods and collected from the polythene ground sheet. Grinding took place in a tungsten carbide hammer mill.

The homogeneity was tested by INAA for a range of elements (Cl, P, S and N as a first test and Br, Co, Cu, Fe, Ba, Mn, Na, Sc, Sm, W, Eu and Cr as a second test) [2] and the homogeneity was found to be sufficient at the level of 100 mg. A separate study was carried out by HNO_3/HCl digestion followed by ICP-AES on Hg. This element may be present in the plants initially as organically bound and as complexed Hg(II) . Due to the drying at elevated temperatures and grinding, a part of this mercury might be converted into elemental mercury which in turn may migrate through the matrix and show preferential sites of adsorption. If a homogeneous distribution of Hg is found, one can assume that the natural speciation of the element is not too much disturbed. The within- and between-bottle homogeneity of Hg was assessed (20 replicate determinations) and no inhomogeneity could be detected at a level of 100 mg. Finally, the between-bottle homogeneity of the Al contents was verified by INAA by 40 determinations. No inhomogeneity could be detected at the 100 mg level, which confirmed the extensive study performed for a range of trace elements.

The materials were dried and kept several hours at 130°C to avoid biological degradation upon storage. The moisture content (2–5%) was considered to be low enough to avoid biological activity in such samples. The stability of the material was checked over four years by analysing the contents of Cd, Hg and Pb at two occasions (1978 and 1982) in the same laboratory, using the same technique. The results demonstrated that the stability of the materials was suitable for using them as CRMs [2].

6.1.3. Certification

Twelve laboratories from seven European countries participated in the certification (see section 6.1.4). The techniques of final determination used are summarised in Table 6.1. The pretreatment methods were digestion with a combination of acids in a pressurised or atmospheric mode, programmed dry ashing, combustion or irradiation with thermal neutrons [2]. A second group of laboratories additionally determined the contents of

TABLE 6.1

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN AQUATIC PLANTS CRMS 060 AND 061 AND OLIVE LEAVES CRM 062

Elements	Techniques of final determination
Cd	DPASV, ETAAS, ICP-AES, RNAA
Cu	DPASV, ETAAS, FAAS, ICP-AES, IDMS, RNAA
Hg	CVAAS, CVAFS, ETAAS, ICP-AES, RNAA
Mn	ETAAS, FAAS, ICP-AES, IDMS
Pb	DPASV, ETAAS, FAAS, IDMS
Zn	FAAS, ICP-AES, INAA, RNAA
Al	DCP-AES, ETAAS, FAAS, ICP-AES, INAA, SPEC, TITR

Al for certification [3]; pretreatment methods were either wet digestion (using mixture of acids, including HF) or fusion, or irradiation with thermal neutrons.

The dry ashing technique was questioned and was not recommended for certification. One laboratory tried to carry out sample pretreatment using this technique and checked the results with a wet digestion; after repeated experiments, the laboratory concluded that a systematic error was occurring with the dry ashing method. The participants recognised that dry ashing may generally lead to erroneous results for the determination of elements which can form volatile compounds which may be the case e.g. for Cd, Pb and Zn. As there was an evidence that the temperature applied in the dry ashing method for CRMs 060 and 061 by the laboratory was too high (leading to low results for Zn), the results were rejected. The certified values (accepted after both technical and statistical evaluations) are given in Table 6.2.

During the course of this certification some laboratories individually determined other elements. These results are given as indicative values in the certification report [2].

6.1.4. Participating laboratories

The preparation of the materials was carried out by the Joint Research Centre in Ispra (Italy). The homogeneity has been verified by the Gesellschaft für Strahlen- und Umweltforschung in Neuherberg (Germany), the Badische Anilin und Sodafabrik (BASF) in Ludwigshafen (Germany) and the Institut für Reinststoffanalyse (Germany). The homogeneity of the Al content was verified by the Energieonderzoek Centrum Nederland in Petten (The Netherlands).

The following laboratories participated in the certification campaign: Centro di Radiochimica ed Analisi per Attivazione, Pavia (Italy); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Gesellschaft für Strahlen- und Umweltforschung, Neuherberg (Germany); Institut Fresenius, Taunusstein (Germany); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Joint Research Centre, Ispra (Italy); Kernforschungsanlage, Jülich (Germany); Laboratory of the Government

TABLE 6.2A

CERTIFIED CONTENTS OF TRACE ELEMENTS IN CRMS 060, 061 AND 062

Element	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
CRM 060		
Cd	2.20	0.10
Cu	51.2	1.9
Hg	0.34	0.04
Mn	1759	51
Pb	63.8	3.2
Zn	313	8
CRM 061		
Cd	1.07	0.08
Cu	720	31
Hg	0.23	0.02
Mn	3771	78
Pb	64.4	3.5
Zn	566	13
CRM 062		
Cd	0.10	0.02
Cu	46.6	1.8
Hg	0.28	0.02
Mn	57.0	2.4
Pb	25.0	1.5
Zn	16.0	0.7

TABLE 6.2B

CERTIFIED CONTENT OF AL IN CRMS 060, 061 AND 062

Aluminium	Certified value (g kg ⁻¹)	Uncertainty (g kg ⁻¹)
CRM 060	4.18	0.12
CRM 061	10.74	0.24
CRM 062	0.448	0.017

Chemist, Teddington (United Kingdom); Landwirtschaftskammer Westfalen Lippe, Joseph-König-Institut, Münster (Germany); National Food Agency, Søborg (Denmark); Office de la Recherche Scientifique et Technique Outre-mer, Bondy (France).

The following laboratories participated in the additional certification: An Forais Taluntais, Wexford (Ireland); Bundesanstalt für Materialforschung und Prüfung, Berlin (Germany); Centro di Radiochimica ed Analisi per Attivazione, Pavia (Italy); CNRS, Service Central d'Analyse, Vernaison (France); Energieonderzoek Centrum Nederland,

Petten (The Netherlands); Gesellschaft für Strahlen- und Umweltforschung, Neuherberg (Germany); INRA, Station de Recherches sur le Sol, la Microbiologie et la Nutrition des Arbres Forestiers, Champenoux (France); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Instituut voor Scheikundig Onderzoek, Tervuren (Belgium); Kernforschungsanlage, Jülich (Germany); Plymouth Polytechnic, Department of Environmental Sciences, Plymouth (United Kingdom); Risø National Laboratory, Isotope Division, Roskilde (Denmark); University of Strathclyde, Department of Pure and Applied Chemistry, Glasgow (United Kingdom)

6.2. TRACE ELEMENTS IN SEA LETTUCE

6.2.1. Introduction

Monitoring trace contaminants in water gives only data on concentration levels at the time of sampling and does not provide suitable information for the study of contamination trends. The total pollution over a period of time, including peak concentrations, and its effect on and uptake by living organisms, can only be obtained by measuring the integrated contents in organisms living in the particular environment. Aquatic plants are frequently analysed since they are considered as good indicators for the contamination of the environment they live in. This section describes the certification of a sea lettuce certified reference material (CRM 279) which has been certified for its trace element contents by BCR in 1987 [4].

6.2.2. Production of the material

The sea lettuce (*Ulva lactuca*) was collected in stagnant saline water in Lake Veere (The Netherlands) near Kwistenburg with a special cutting and grab machine mounted on ship board. The material was cut at a depth of a maximum of 1 m below the water surface to minimise the amount of adhering sand particles in the final product. Possibly adhering particles were washed off using water from the lake. Drying took place in a drum with hot air for about 1 h. The loss of material was about 93%.

After a coarse grinding (to a particle size of 2–10 mm) in a stainless steel mill, the material was packed in polythene lined containers and shipped to the Joint Research Centre of Ispra (Italy) for further processing. The material was then ground in a tungsten carbide mill. A fraction of the particles of 90–200 µm was sieved out; this fraction was chosen in order to eliminate possible fine clay-like particles.

Mixing was performed during two weeks under dry air (which was replaced at least twice a day) in a special mixing polythene lined drum. After this period, bottling was carried out in units of ca. 35 g. After each batch of 40 bottles the content of the drum was mixed again for 30 minutes. One bottle was selected randomly out of each batch and set aside for the homogeneity study.

The homogeneity of the material for its matrix composition was verified for the elements Cl, S, Ca, K and P. Chlorine was determined after an oxygen flask combustion by an argentometric titration with potentiometric detection; sulphur was determined

by acidimetric titration with coulometric hydroxyl generation after an oxygen flask combustion. Dry ashing up to 550°C followed by a treatment of the residue with $\text{HClO}_4/\text{HNO}_3/\text{HF}$ was chosen for the ICP-determination of Ca, K and P. The matrix homogeneity was considered to be good at levels of intake of 100 mg and above. Further studies with sample intakes of 25–500 mg were carried out for trace elements, using ICP-AES, ETAAS or INAA as final detection techniques as described elsewhere [5,6]. The measurements were based on 20 replicate determinations. The methodological CV of INAA was based on the counting statistics only (possible random errors resulting from experimental parameters such as irregular loading of the vial, different distances to counting systems etc. were neglected); the methodological CV of ICP and ETAAS was based on 10 repeated measurements of an acidified solution; it does not take into account the contribution of the digestion, the variations in the digest solutions and further handling, to the overall uncertainty. The CVs were not significantly different for the various elements tested (Ca, Cd, Cr, Fe, Mg, Mn, Na, Ni, V, Zn), i.e. the CVs of the methods, within- and between-bottle were comparable. It was concluded that the material is homogeneous at a level of 100 mg intake and above [4].

Stability testing was performed at -20 , $+20$ and $+40^\circ\text{C}$ over a period of 12 months both for matrix components (C, H, N, P, Mn, Na, K, Cl) and trace elements (As, Br, Co, Cr, Hg, I, Se, Zn). Measurements were carried out at the moment of storage and after 2 weeks, 3, 6 and 12 months. C, H and N were determined by automatic element analyser based on combustion, chromatographic separation and catharometric measurement; P was determined by combustion followed by ICP; and Mn, Na and Cl were determined by INAA. With respect to the matrix composition, there was no significant changes in the major and minor elements nor change in either the physical appearance or smell of the material. In addition to this study, elements such as As, Se, Hg, Br, Zn, Cr which are volatile in the free state or may form volatile compounds during storage were also monitored over the stability testing period. Hg was determined by CVAAS whereas I, Br, Cr, Zn, Co, Se and As were determined by INAA. The results showed that no detectable change of element contents could be detected under any of the conditions tested; consequently, it was concluded that the stability of the material was suitable for use as a certified reference material [4].

6.2.3. Certification

Twelve laboratories from eight European countries participated in the certification (see section 6.2.4). The techniques of final determination used are summarised in Table 6.3. The pretreatment steps were digestion with a combination of acids in a pressurised or atmospheric mode, programmed dry ashing, combustion or irradiation with thermal neutrons [4].

For arsenic, one laboratory using HICP performed a pre-reduction to As(III) with KI/ascorbic acid of which the yield depends strongly on the composition of the digest solution; calibration by standard additions of As(V) was therefore recommended.

TABLE 6.3

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN SEA LETTUCE CRM 279

Elements	Techniques of final determination
As	HAAS, HICP, RNAA, SPEC, ZETAAS
Cd	DPASV, ETAAS, IDMS
Cu	DPASV, ETAAS, FAAS, ICP-AES, IDMS, RNAA, ZETAAS
Pb	DPASV, ETAAS, FAAS, IDMS, ZETAAS
Se	FLUOR, HAAS, HICP, INAA, RNAA
Zn	DPASV, FAAS, ICP-AES, IDMS, INAA, RNAA

Another laboratory using RNAA had decided to reduce the irradiation time to the minimum in order to avoid volatilisation of As upon irradiation which subsequently caused a loss upon opening the vial after irradiation; the laboratory had a lower activity of As which led to a higher standard deviation.

Various reasons for the discrepancy between results of Cd determinations by DPASV were discussed: presence of high amounts of nitrate (causing a change in the peak shape), incomplete digestion, inadequate pH-value and others. The variances obtained with the technique were not significantly different from one laboratory to another and none of the suggested causes could explain the differences in the results; all the results were therefore kept for certification.

For copper, the relatively and unusually high content of I (about 160 mg kg⁻¹) had caused difficulties both in electrochemical analysis (e.g. complexation) and in spectrometric analysis (e.g. volatility of iodides in volatilisation step in ETAAS); measures had to be taken to remove or bind the iodide (e.g. by complexation, precipitation or sulphuric acid treatment).

Doubts were expressed on DPASV sets for Pb which were suspected to be due to iodide interferences.

A short irradiation time in RNAA for Se was found to be insufficient to achieve accurate and precise results.

Discrepancies in INAA sets of data were noticed for Zn. Discussions focused on the irradiation position of the samples in the rotating rig, the primary calibrants used, possible shelf shielding in the calibrant, the resolution of the gamma-energies in the detector and the counting geometry; none could explain the discrepancies.

All data accepted after technical scrutiny were statistically evaluated; none were rejected on a statistical basis. The certified values are given in Table 6.4. During the course of this certification some laboratories individually determined other elements. These results are given as indicative values in the certification report [4].

The comparison of methods for some elements showed that no particular bias could be attributed to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 6.5)

TABLE 6.4

CERTIFIED CONTENTS OF TRACE ELEMENTS IN SEA LETTUCE CRM 279

Element	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
As	3.09	0.20
Cd	0.274	0.022
Cu	13.14	0.37
Pb	13.48	0.36
Se	0.593	0.032
Zn	51.3	1.2

TABLE 6.5

COMPARISON OF METHODS FOR As, Cd, Cu, Pb, Se AND Zn IN CRM 279 (SEA LETTUCE)

Element	Techniques	Number of labs	CV (%) (*)	CV (%) (**)
As	RNAA	3	7.9	3.2
	HAAS	5	12.5	
Cd	DPASV	6	14.7	0.3
	AAS	3	10.4	
	IDMS	2	5.2	
Cu	DPASV	4	4.7	2.9
	AAS	5	7.3	
	IDMS	2	1.5	
Pb	DPASV	5	9.3	1.2
	AAS	5	4.1	
Se	FLUOR	2	0.04	1.2
	NAA	3	11.2	
	HAAS	4	8.0	
Zn	DPASV	2	0.5	1.1
	NAA	3	5.8	
	AAS	4	3.9	
	ICP-AES	2	1.7	
	IDMS	2	2.8	

(*) CV between means of laboratories with the same technique

(**) CV between means of different techniques

6.2.4. Participating laboratories

The preparation of the material was carried out by the Delta Instituut voor Hydrobiologisch Onderzoek in Yerseke (The Netherlands) and the Joint Research Centre in Ispra (Italy). The homogeneity has been verified by the CNRS, Service Central d'Analyse in Vernaison (France) and the Gesellschaft für Strahlen- und Umweltforschung

in Neuherberg (Germany). Finally, the stability study has been performed by the Energieonderzoek Centrum Nederland in Petten (The Netherlands).

The following laboratories participated in the certification campaign: An Forais Taluntais, Wexford (Ireland); Centro di Radiochimica ed Analisi per Attivazione, Pavia (Italy); CNRS, Service Central d'Analyse, Vernaison (France); Delta Instituut voor Hydrobiologisch Onderzoek, Yerseke (The Netherlands); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Gesellschaft für Strahlen- und Umweltforschung, Neuherberg (Germany); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Joint Research Centre, Ispra (Italy); Kernforschungsanlage, Jülich (Germany); The Macaulay Land Use Research Institute, Aberdeen (United Kingdom); Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven (The Netherlands); Risø National Laboratory, Isotope Division, Roskilde (Denmark).

6.3. TRACE ELEMENTS IN RYE GRASS

6.3.1. Introduction

Grass and clover for animal feed are routinely analysed to determine elements of nutritive quality (e.g. Ca, Mg, K, P, N, Mn etc.) and of potential risk (e.g. Cd, Hg). The results of determination of nutritive elements are used in the design of a well-balanced diet. The determination of the dangerous elements is necessary to avoid affecting the health of domestic animals which would lead to a lower production (economic loss) or even could cause damage to the consumer of animal products (milk, meat etc.). The rye grass material described in this chapter was produced for the quality control of determinations of important heavy elements, either of dangerous nature (e.g. Cd, Pb, Hg, Cu, Zn) or their role in animal nutrition (e.g. Se, Mo) [7,8].

6.3.2. Production of the material

A semi-rural site on the western outskirts of Aberdeen was chosen to grow the grass on a pedologically freely drained soil [7]. The rye grass was harvested and cut by hand with carbon-steel sheep shears about 5 cm above the soil. The grass was then placed directly in cleaned polyethylene sacks, avoiding any contact between grass and soil in order to prevent contamination. The sealed polyethylene sacks were transferred immediately to a cold store (4°C) to await drying.

The purity of the grass stand was about 99%; all other species were rejected when cut. The grass consisted entirely of leaf material without heading; it was about 20–30 cm tall. Drying took place in a ventilated oven at about 50°C. The grass came in contact only with cast iron and was spread on washed filter paper.

Dried material was sent to the Joint Research Centre of Ispra in double-layer polythene bags where the material was ground by ball-milling (zirconia) and sieved to pass apertures of 125 µm. The resulting material was collected in a 180 L PTFE-lined mixing drum and homogenised for two weeks. At regular intervals the gas in the drum was replaced by dry argon.

Portions of ca. 30 g were transferred into cleaned glass bottles provided with a screw cap and a plastic insert. A PTFE ball was added in each bottle to allow the user to re-homogenise the material before opening.

After filling 40 bottles, the drum was again closed, flushed with dry argon and the contents were re-mixed for about 15 min. Then the filling of another 40 bottles was performed. Out of every batch of 40 bottles, one was selected randomly and set aside for further homogeneity studies.

The within- and between-bottle homogeneity was studied with instrumental neutron activation analysis (INAA) on samples of 50 to 250 mg. Care was taken that all samples received the same integrated flux (turntable in reactor). The general procedure followed has been described in the literature [5,6]. A more detailed study was carried out by INAA to demonstrate the necessity to re-homogenise the contents of the bottle before taking a sample; results are summarised in Table 6.6. Bottles 3 and 4 were analysed as received; bottles 1, 2 and 5 were re-homogenised by manual shaking prior to taking sub-samples. The results demonstrated a good homogeneity at levels above 100 mg, provided that re-homogenisation is carried out, i.e. the coefficient of variations (CV) of the method, and the CVs within- and between-bottles were in the same range (3.5 to 6.5%) for all elements [7].

Previous thorough stability experiments on plant materials, which were prepared with the same equipment and following the same procedure, have demonstrated a good stability of the obtained material [9]. This good stability for dried plant materials (e.g. hay) can be achieved as long as the moisture content is sufficiently low (e.g. below 10%); biological degradation may start at higher moisture contents. To verify the expected stability, the contents of trace elements of interest were determined by HICP (As and Sb), ICP-AES (B), DPASV (Cd and Pb), and ETAAS (Co, Mo and Ni) in the material kept at ambient temperature in the laboratory in closed bottles over a period of 18 months. The results demonstrated that the material was stable and suited for use as a reference material [7].

TABLE 6.6

ADDITIONAL HOMOGENEITY STUDY OF CRM 281 (RYE GRASS) BY INAA FOR Cr AT THE 100 MG LEVEL (5 REPLICATES)

Bottle	Within-bottle CV (%)	Between-bottle CV (%)
1°	4.5	
2°	5.6	
3*	11.1	5.5
4*	10.6	
5**	8.4	

° Bottles reshaken for 2 min

* Bottles not reshaken

** Bottles reshaken for 30 s

6.3.3. Certification

Twenty-four laboratories from nine European countries participated in the certification (see section 6.3.4). The techniques of final determination used are summarised in Table 6.7. The pretreatment consisted of digestion with a combination of acids in a pressurised or atmospheric mode, programmed dry ashing, combustion or irradiation with thermal neutrons [7].

The SETAAS technique had high background values for arsenic and antimony, which could not be reduced sufficiently even after a slightly prolonged ashing. A prolonged ashing might cause losses of volatile As-compounds and could, therefore, not be considered. This technique is probably not suited for such a material and the results were consequently withdrawn.

The determination of boron is as yet not fully in control in many laboratories, which is an indication that the interest in this element has increased only recently in most environmental laboratories. This situation was reflected in the spread of the results, which nevertheless seemed to correspond to the state of the art at the moment. Some sources of errors detected were e.g. high limits of detection for INAA, memory effects in ICP-MS and calibration error. ICP-AES is a widely used technique to determine boron; however, the technique must be applied with great care. Typical problems encountered in such a matrix are iron interferences at the most suited boron-line and ashing procedure causing a high and irreproducible blank.

All results provided for Cd, Cu, Hg, Mo, Mn and Pb were found to be technically

TABLE 6.7

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN RYE GRASS CRM 281

Elements	Techniques of final determination
As	ETAA, HAAS, HICP, ICP-MS, INAA, SETAAS
B	ICP-AES, ICP-MS, INAA, MS, SDCP, SPEC
Cd	DPASV, ETAAS, ICP-AES, MS, ZETAAS
Cu	DPASV, ETAAS, FAAS, ICP-AES, ICP-MS, INAA, MS, ZETAAS
Hg	CVAAS, INAA, MIP-AES
Mn	FAAS, ICP-AES, INAA, ZETAAS
Mo	ETAAS, ICP-AES, ICP-MS, INAA
Ni	ADPV, DPASV, ETAAS, FAAS, ICP-AES, INAA, ZETAAS
Pb	DPASV, ETAAS, FAAS, ICP-MS, MS, ZETAAS
Sb	HAAS, HICP, ICP-MS, INAA, SETAAS
Se	FLUOR, ETAAS, HAAS, INAA
Zn	DPASV, FAAS, ICP-AES, INAA, MS

ADPV: Adsorption differential pulse voltammetry

SETAAS: slurry ETAAS

SDCP: direct slurry DCP

acceptable for certification, except two sets for Cd and Mo which were too close to the ICP-AES limit of determination, and one set for Pb (high blanks in ICP-MS).

Interferences were noted for Ni determination by INAA. The normal Ni-peak had a strong interference in the gamma-spectrum; therefore the laboratory decided to measure the nickel content via the ^{58}Co -peak ($^{58}\text{Ni}(\text{n,p})^{58}\text{Co}$) which is not very sensitive and too close to detection limits.

A high standard deviation was observed for the FLUOR determination of Se, which was attributed to the digestion procedure requiring extra effort (longer digestion time than usual and addition of extra reagents).

Calibration errors justified that two sets of Zn results be withdrawn (no verification of commercial calibrant nor matrix-matching, and use of a CRM for calibration).

The certified values are presented in Table 6.8 together with their calculated uncertainties. During the course of this certification some laboratories individually determined other elements. These results are given as indicative values in the certification report [7].

The comparison of methods for some elements did not allow us to detect any bias due to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 6.9).

6.3.4. Participating laboratories

The preparation of the material was carried out by the Macauley Land Use Research Institute in Aberdeen (United Kingdom) and the Joint Research Centre in Ispra (Italy). The homogeneity has been verified by the Centro di Radiochimica ed Analisi per Attivazione of the University of Pavia (Italy) and the Energieonderzoek Centrum Nederland in Petten (The Netherlands); finally, the stability study has been performed by the CNRS, Service Central d'Analyse in Vernaison (France).

TABLE 6.8

CERTIFIED CONTENTS OF TRACE ELEMENTS IN RYE GRASS CRM 281

Element	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
As	0.057	0.004
B	5.9	0.7
Cd	0.120	0.003
Cu	9.65	0.38
Hg	0.0205	0.0019
Mn	81.6	2.6
Mo	0.84	0.06
Ni	3.00	0.17
Pb	2.38	0.11
Sb	0.047	0.005
Se	0.028	0.004
Zn	31.5	1.4

TABLE 6.9

COMPARISON OF METHODS FOR TRACE ELEMENTS IN RYE GRASS CRM 281

Element	Techniques	Number of labs	CV (%) (*)	CV (%) (**)
As	RNAA	4	9.4	4.9
	HAAS	5	11.4	
B	ICP-AES	4	16.8	8.8
	SPEC	2	5.2	
Cd	DPASV	3	3.1	1.8
	ZETAAS	2	4.2	
Cu	IDMS	2	0.3	3.1
	DPASV	3	3.5	
	FAAS	4	8.7	
	ICP-AES	2	6.4	
Hg	IDMS	2	6.5	3.4
	RNAA	3	15.3	
	CVAAS	7	11.5	
	FAAS	5	6.0	
Mn	ICP-AES	3	3.1	2.1
	RNAA	3	8.9	
Mo	ETAAS	3	7.5	3.5
	ICP-MS	2	7.6	
Ni	DPASV	2	14.3	3.3
	ZETAAS	7	8.9	
	FAAS	2	1.0	
	ICP-AES	2	5.7	
Pb	DPASV	4	8.7	2.0
	ZETAAS	5	10.3	
	FAAS	3	11.1	
	ICP-MS	2	0.2	
Sb	IDMS	2	5.0	5.2
	INAA	4	14.0	
Se	RNAA	2	21.0	10.9
	INAA	2	16.3	
Zn	RNAA	2	29.0	2.4
	HAAS	4	15.6	
	INAA	3	6.8	
	FAAS	4	10.9	
	ICP-AES	2	8.8	
	IDMS	2	8.0	

(*) CV between means of laboratories with the same technique

(**) CV between means of different techniques

The following laboratories participated in the certification campaign: An Forais Taluntais, Wexford (Ireland); Arbeitsgruppe Systemforschung, Universität Osnabrück (Germany); Centro Regionale per l'Incremento della Vitivinicoltura Frutticoltura e

Cerealicoltura, Pavia (Italy); Centro di Radiochimica ed Analisi per Attivazione, Pavia (Italy); CNRS, Service Central d'Analyse, Vernaison (France); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Gesellschaft für Strahlen- und Umweltforschung, Neuherberg (Germany); INRA, Station d'Agronomie, Villenave d'Ornon (France); Instituut voor Nucleaire Wetenschappen, Rijksuniversiteit, Gent (Belgium); Institut für Spektrochemie, Dortmund (Germany); Isotopcentralen, Copenhagen (Denmark); JRC Central Bureau for Nuclear Measurements, Geel (Belgium); Kernforschungsanlage, Jülich (Germany); Kon. Shell Laboratorium, Amsterdam (The Netherlands); Landwirtschaftskammer Westfalen-Lippe, Münster (Germany); The Macauley Land Use Research Institute, Aberdeen (United Kingdom); Monitor, Analytisch Laboratorium, Amersfoort (The Netherlands); National Food Agency, Søborg (Denmark); Plymouth Polytechnic, Department of Environmental Sciences, Plymouth (United Kingdom); Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven (The Netherlands); Studiecentrum voor Kernenergie, Mol (Belgium); TNO Technology for Society, Delft (The Netherlands); Universidad Complutense, Departamento de Química Analítica, Madrid (Spain).

6.4. TRACE ELEMENTS IN HAY POWDER

6.4.1. Introduction

In order to improve the state of health and growth of domestic animals, their diet must be well balanced, therefore basic feed often needs to be enriched with respect to trace and minor elements. Such additions are made on the basis of results of analysis of the raw material, e.g. hay. A hay powder reference material has been considered necessary as being complementary to the rye grass material described in section 6.3. The EC Directive 79/373/EEC prescribes the determination of the nutrients Ca, P and Mg; S and N are also important elements since they are used as indicators of the availability of proteins; finally the administration of iodine to growing animals requires that this element be carefully monitored. Consequently, the hay powder CRM 129 was certified for its contents of Ca, K, Mg, P, S, Zn, I, N and Kjeldahl-N [10,11].

6.4.2. Production of the material

The hay powder was obtained from a dried grass cultivated on a selected and well prepared soil (granitic and gneissic origin) sprayed with herbicides and fertilised with P, K and high amounts of nitrate (ca. 100 kg.ha⁻¹). The grass was cut by hand with carbon steel sheep shears in fine weather, 5 cm above the soil. Any contact between grass and soil was avoided. The grass consisted entirely of leaf material without heading. It was dried immediately after harvesting in a ventilated oven at about 40°C. Then it was sent the Central Bureau of Nuclear Measurements in Geel (Belgium) in polythene bags for the final preparation.

The material was dried at 100°C with pulsed hot air in a stainless steel container and ground in a tungsten carbide grinder to pass apertures of 120 µm. The resulting material

was homogenised in a special PTFE-lined drum for 30 min. Portions of about 25 g were transferred into clean brown glass bottles provided with a screw cap and a plastic insert. After filling 40 bottles the contents of the drum were re-mixed for 15 minutes. Then the filling of another 40 bottles was performed; one bottle was set aside out of every batch of 40 bottles for homogeneity studies.

The homogeneity study of the material was studied for Ca, Fe, K, Mg and Na at the 100 mg level, and at the 2 mg level for N. The technique of determination was based on digestion with perchloric/nitric acid mixture under reflux for 5 h. After HF addition, the digest was evaporated to dryness and the residue was dissolved in diluted HCl. This solution was analysed for Ca, Fe, K, Mg and P by ICP-AES. For the nitrogen determination, about 2 mg were combusted in a He/O₂ stream; N-containing combustion products were converted into N₂ over Cu and CuO and all gaseous combustion products were separated by gas chromatography and catharometric detection. The variability of the ICP-AES measurement typically ranged from 0.5 to 1% as measured by 10 replicate analysis of the same solution. It is well recognised that the variability from the digestion can be up to 2–5% [12]. In the case of the hay powder the variability obtained in the within- and between-bottle homogeneity study ranged from 0.9 to 3.5% [1], which reflected totally the variability to be expected from the method of measurement. This allowed us to conclude that the material is homogeneous at the 100 mg level and above.

Previous stability experiments on plant materials have demonstrated a good stability of this type of material, providing that the moisture content is sufficiently low (i.e. below 10%); biological degradation may start at higher moisture content. To verify this expected stability, the contents of six elements were determined in the material kept at –20, +20 and +40°C at intervals of 4, 7, 11 and 14 months. Apparent variations of the values obtained were rather due to the long-term variability of the analytical method than to instability. Even at prolonged storage at +40°C losses could not be detected and the material was hence considered to be suitable for use as a reference material [10].

6.4.3. Certification

Sixteen laboratories from ten European countries participated in the certification (see section 6.4.4). The techniques of final determination used are summarised in Table 6.10. The pretreatment was digestion with a combination of acids in a pressurised or atmospheric mode, programmed dry ashing, combustion or irradiation with thermal neutrons [10].

At several occasions the soil in which the grass was cultivated was fertilised with large amounts of nitrates (ca. 180 kg ha⁻¹) up to one month before the harvest. As a consequence, the nitrate content of the material is high (approx. 3 g kg⁻¹) which is the reason for the difference between results of Kjeldahl-N and total-N determinations. The Kjeldahl method does not reduce nitrates at low temperatures, so the results of the total-N determinations are therefore significantly higher. For this reason, it was decided to certify both the total-N content and the Kjeldahl-N content as the latter is of great importance for the practical use of the material (Kjeldahl-N can in principle be bio-converted in the living body into protein).

The certified values are presented in Table 6.11. Some participants determined other

TABLE 6.10

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN HAY POWDER CRM 129

Elements	Techniques of final determination
Ca	FAAS, ICP-AES, IDMS, INAA, TITR
I	INAA, RNAA, SPEC
K	FAAS, FAES, ICP-AES, INAA
Mg	FAAS, ICP-AES, IDMS
N	GC-AAS, CS-CA, CS-VO
Kjeldahl-N	KJEL, IDMS
P	ICP-AES, RNAA, SPEC, TITR
S	ICP-AES, IC, IDMS, SPEC
Zn	FAAS, ICP-AES, INAA, RNAA

CS-CA: Chemical separation and catharometric detection

CS-VO: Chemical separation and gas volumetric measurement

GC-CA: Gas chromatography and catharometric detection

TABLE 6.11

CERTIFIED CONTENTS OF TRACE ELEMENTS IN HAY POWDER CRM 129

Element	Certified Value	Uncertainty	Unit	Number of accepted sets
Ca	6.4	0.1	g kg^{-1}	13
K	33.8	0.8	g kg^{-1}	9
Mg	1.45	0.04	g kg^{-1}	8
P	2.36	0.07	g kg^{-1}	8
N	37.2	0.5	g kg^{-1}	7
Kjeldahl-N	34.2	0.4	g kg^{-1}	4
S	3.16	0.04	mg kg^{-1}	8
I	0.167	0.024	mg kg^{-1}	5
Zn	32.1	1.7	mg kg^{-1}	6

elements in the course of the certification campaign which are given in the certification report [10].

The comparison of methods for some elements did not allow us to detect any bias due to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 6.12).

6.4.4. Participating laboratories

The preparation of the material was carried out by the Macauley Land Use Research Institute in Aberdeen (United Kingdom) and the Central Bureau for Nuclear Measurements in Geel (Belgium). The homogeneity has been verified by the CNRS, Service

TABLE 6.12

COMPARISON OF METHODS FOR Ca, K, Mg AND N IN HAY POWDER CRM 129

Element	Techniques	Number of labs	CV (%) (*)	CV (%) (**)
Ca	FAAS	4	3.2	2.0
	ICP-AES	3	2.0	
	INAA	3	0.7	
K	ICP-AES	3	2.9	1.5
	INAA	3	3.8	
Mg	FAAS	4	1.7	1.1
	ICP-AES	3	4.7	
N	GC-CA	4	1.3	0.8
	CS-CA	3	0.7	

(*) CV between means of laboratories with the same technique

(**) CV between means of different techniques

Central d'Analyse in Vernaison (France). The stability has been verified by the Universidad Complutense, Departamento de Química Analítica, in Madrid (Spain).

The following laboratories participated in the certification campaign: An Forais Taluntais, Wexford (Ireland); Aristotle University, Laboratory of Analytical Chemistry, Thessaloniki (Greece); CNRS, Service Central d'Analyse, Vernaison (France); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Gesellschaft für Strahlen- und Umweltforschung, Neuherberg (Germany); Istituto Superiore di Sanità, Roma (Italy); Instituut voor Nucleaire Wetenschappen, Rijksuniversiteit, Gent (Belgium); Institut de Recherches Chimiques, Tervuren (France); Landesanstalt für Ökologie, Recklinghausen (Germany); NCR Demokritos (Greece); Novo Industri A/S, Bagsvaerd (Denmark); Risø National Laboratory, Roskilde (Denmark); The Queen's University, Department of Chemistry, Belfast (United Kingdom); TNO Instituut voor Toegepaste Chemie, Zeist (The Netherlands); Universidad Complutense, Departamento de Química Analítica, Madrid (Spain).

6.5. TRACE ELEMENTS IN WHITE CLOVER

6.5.1. Introduction

Clover analyses are routinely performed for monitoring purposes, mapping and risk assessment. In particular, clover and grass for animal feed are analysed to determine elements of nutritive importance (e.g. Se, Mo) and of potential toxicity (e.g. As, Co). The results of the determination of nutritive elements are used to improve the state of health and growth of domestic animals. Their diet must be well balanced and therefore basic feed often needs to be enriched with respect to trace and major elements. The determination of toxic elements is necessary to avoid health damage to the animals,

which would lead to a lower production (economic losses) or even could cause a hazard to the consumer of animal products, e.g. milk, meat. To control the quality of determinations of various elements and consequently the quality of feedstuffs, various certified reference materials are required. The white clover material (CRM 402) presented in this section has been certified for elements of most interest for nutritional purpose (As, Co, Mo and Se) [13,14].

6.5.2. Production of the material

The white clover was grown and harvested on a field with Se-rich and poorly drained soils. The collected material was dried at 70°C in aluminium-lined ovens with clean paper on the shelves. The grinding of the dried material was carried out in two steps using a cast-iron rotor beater mill. A cutting mill was used for coarse and fine grinding of soft to medium-hard and fibrous materials in order to obtain particles passing through a sieve with 2-mm apertures. The powder had a strong tendency to agglomerate during fine grinding because of the moisture content of the material (ca. 5.5%). The second grinding step was therefore possible only after a further 2 h drying of the powder at 60°C in air. Previous experiments had demonstrated that Se losses were not to be expected at this temperature. The material obtained was sieved through a polypropylene-carbon sieve with 120- μ m apertures.

The powder obtained was dried again for 2 h at 60°C and homogenised for 24 h in a PTFE-lined mixing drum. The powder was then transferred into a dry-air glove box connected to a gas purification system. At this stage, the moisture content of the white clover was below 3% (w/w). Clean, brown-glass bottles provided with a PTFE ball for rehomogenisation were filled with ca. 25 g of material and closed with plastic inserts and screw-caps.

The homogeneity of the material was verified by two independent studies; firstly by the determination of Co, Mo and Se on intakes of 100 mg, and secondly by the determination of B, Ba, Ca, Cr, K, Mg, Mn, Na, Ni, P, S, Sr and Zn on intakes of 100 mg. In the first study, analyses were performed by ETAAS (Mo), HGAAS (Se) and ADPCSV (Co) after digestion of the material with HNO₃ and HF at 140°C for 6 h in a PTFE bomb. In the second study, analyses were done by ICPAES after pressurised digestion with HNO₃-HCl (3:1) in a quartz vessel at 170°C for ca. 8 h. The results showed that the material is homogeneous at the level of 100 mg and above.

Three series of bottles were kept at respectively -20, +20 and +40°C over a period of 12 months, and minor (Ca) and trace elements (Co, Mo, Ni and Se) were determined in five replicates. The material was found to be stable enough to be used as a reference material [13].

6.5.3. Certification

Twenty-two laboratories from ten European countries participated in the certification (see section 6.5.4). The techniques of final determination used are summarised in Table 6.13. The pretreatment was digestion with a combination of acids in a pressurised or

TABLE 6.13

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN WHITE CLOVER CRM 402

Element	Techniques used in certification
As	HGAAS, HICP, RNAA, SPEC
Co	ICP-MS, INAA, RNAA
Mo	DCP, EDXRF, ETAAS, ICP-AES, ICP-MS, RNAA, ZETAAS
Se	EDXRF, FLUO, HGAAS, HICP, RNAA, ZETAAS

atmospheric mode, programmed dry ashing, combustion or irradiation with thermal neutrons [13].

It is well known that botanical materials may contain various soil and/or mineral fractions, and may therefore be difficult to dissolve [15,16]. Since the white clover material contains silicates, it was necessary to treat the material with HF to ensure complete digestion and recovery of the total metal content. Results obtained with destructive methods without using HF were therefore withdrawn, unless the laboratory could prove that the residue of the digest did not contain the elements determined. Neutron activation analysis was an important method for identifying the losses due to incomplete digestion.

ETAAS using deuterium as background correction was not considered to be suitable for the determination of Co since the absorption line of this element is close to the end of the deuterium continuum. A general remark was that AAS showed poor agreement for Co in this material. INAA and ICP-MS proved to be better suited for certification in this instance.

Matrix effects were suspected to cause systematic errors in the determination of Mo and the use of standard addition procedures for calibration was strongly recommended.

It was systematically observed that the results from laboratories using open digestion systems were low whereas the highest values corresponded to closed digestion procedures; this demonstrated that losses of Se are likely to have occurred. In addition, a large spread and a lack of overlap between all INAA results were observed which justified withdrawing this technique for the certification of Se in this material.

The certified values are presented in Table 6.14. Indicative values are given for information in the certification report [13].

The comparison of methods for some elements showed that no particular bias could be attributed to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 6.15)

6.5.4. Participating laboratories

The sample collection and preparation has been carried out by the Agriculture and Food Development Authority in Wexford (Ireland) and the Institute for Reference

TABLE 6.14

CERTIFIED CONTENTS OF As, Co, Mo AND Se IN WHITE CLOVER CRM 402

	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	Number of accepted sets of results, p
As	0.09	0.010	15
Co	0.178	0.008	7
Mo	6.93	0.19	13
Se	6.70	0.25	15

TABLE 6.15

COMPARISON OF METHODS FOR As, Mo and Se IN WHITE CLOVER CRM 402

Element	Techn. of final determination	CV (%) between means of lab. with the same technique	nr of sets of results	CV (%) between means of diff. techniques
Arsenic	HGAAS	20.9	5	9.2
	HICP	28.6	6	
	RNAA	9.4	4	
Molybdenum	ICP-AES	3.2	5	3.0
	RNAA	2.3	3	
Selenium	HGAAS	6.0	3	0.7
	HICP	7.3	4	
	RNAA	9.6	4	

Materials and Measurements (Belgium); the homogeneity and stability studies have been performed by the Labor für Spurenanalytik in Bonn (Germany) and the GSF-Forschungszentrum für Umwelt und Gesundheit in Oberschleißheim (Germany).

The following laboratories participated in the certification campaign: An Forais Taluntais, Wexford (Ireland); Aristotelian University, Laboratory of Analytical Chemistry, Thessaloniki (Greece); Centre de Recherches Forestières, Nancy (France); Ecole Européenne des Hautes Etudes des Industries Chimiques, Strasbourg (France); Energieonderzoek Centrum Nederland, Petten (The Netherlands); GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Ökologische Chemie, Oberschleißheim (Germany); Istituto Superiore di Sanità, Rome (Italy); Institut National de Recherches Agronomiques, Villenave d'Ornon (France); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Institut de Recherches Chimiques, Tervuren (Belgium); Labor für Spurenanalytik, Bonn (Germany); Laboratory of the Government Chemist, Teddington (United Kingdom); Landesanstalt für Ökologie, Recklinghausen (Germany); Natural Environmental Research Council, Swindon (United Kingdom); NCR

Demokritos, Agia Paraskevi, Attikis (Greece); Rijksinstituut voor de Volksgezondheid, Bilthoven (The Netherlands); Risø National Laboratory, Roskilde (Denmark); Service Central d'Analyse, CNRS, Vernaison (France); Universidad de Barcelona, Departamento de Química Analítica, Barcelona (Spain); Universidad Complutense, Departamento de Química Analítica, Madrid (Spain); Università degli Studi di Pavia, Centro de Radiochimica, Pavia (Italy); Universität Ulm, Sektion Analytik und Höchstreinigung, Ulm (Germany).

6.6. TRACE ELEMENTS IN BEECH LEAVES AND SPRUCE NEEDLES

6.6.1. Introduction

Some years ago the first reports appeared on forest die-back in heavily polluted areas. The impact of this information drew the attention on the situation of local forests, which encouraged research on acid deposition in many countries. The quantitative evaluation of damage and the monitoring of forest quality are difficult. Most theories have been based on visual inspections; however, trends, actual damage and local effects can only be estimated on the basis of scientific measurements, enabling to make correlations between damage and measured pollutant concentrations. A prerequisite for this evaluation is the availability of representative CRMs. The BCR has hence developed for this purpose two reference materials, namely beech leaves and spruce needles, which have been certified for nutrients (e.g. Ca, Mg, K, P and N) and indicator elements (e.g. S, Cl, Al and Mn) [17–19].

6.6.2. Production of the materials

During sunny days in August beech leaves and in October, one year old spruce needles were collected in forests near Paderborn (Germany). This area was not too heavily affected by acid deposition damages. It lies at a distance of 100 km from a main industrial complex. A sufficient amount of trees were cut. Needles and leaves were manually picked from the side of the tree that did not touch the ground. The absence of adhering soil was visually verified. The materials were not washed in order to avoid losses of elements. Any rests of branches were removed and the material was freeze-dried and sent the EC Joint Research Centre of Ispra (Italy). The material was ground by ball-milling (zirconia mill) and sieved to pass apertures of 125 µm. The resulting material was homogenised for two weeks in a PTFE-lined mixing drum. At regular intervals the gas in the drum was replaced by dry argon. Fifty bottles were filled, each containing about 30 g material. The remaining powder was homogenised again; of each of these 50 bottles one was randomly selected and set aside for homogeneity studies. This procedure was repeated until 1000 bottles of each reference material were filled. A PTFE ball was added in each bottle to facilitate the re-homogenisation of the materials prior to use.

The homogeneity was studied at the 100 mg level. The techniques applied were acid digestion followed by AAS detection (Ca, K, Mn and Fe), Kjeldahl type digest with

spectrophotometric detection (P and N), combustion in O₂ followed by ion selective electrode (F), and pelletising followed by XRF or combustion in O₂ followed by titration (S and Cl). Higher CVs obtained for spruce needles in comparison with beech leaves reflected the analytical difficulties (the contents of the elements are lower than for beech leaves). It could, however, be concluded that both materials are sufficiently homogeneous at the 100 mg level.

The contents of B, Mg, Ca, P (ICP-AES after HClO₄/HF/HNO₃ digestion) and S (IC after O₂ combustion) were determined after one year storage at ambient temperature. There was no significant change in the content of the elements mentioned. The conclusion was drawn that the stability under normal storage conditions is sufficiently good to allow the use of the materials as CRMs.

6.6.3. Certification

Twenty three laboratories from eleven European countries participated in the certification campaign (see section 6.6.4). Table 6.16 summarises the different techniques of final determination used by these laboratories. Pretreatment techniques were digestions with combination of acids including HClO₄ and HF, in a pressurised or atmospheric mode, Kjeldahl pretreatment, programmed dry ashing and oxygen combustion. Other methods hardly used any pretreatment (e.g. INAA).

Results of the different laboratories and methods were generally in good agreement and the technical discussion did not identify any particular bias. The certified values are listed in Table 6.17. Indicative values are given in the certification report [17].

The comparison of methods for some elements did not allow us to detect any bias due to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 6.18).

TABLE 6.16

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN BEECH LEAVES CRM 100 AND SPRUCE NEEDLES CRM 101

Element	Techniques used in certification
Al	ETAAS, ICP-AES, INAA, SPEC
Ca	FAAS, ICP-AES, IDMS, INAA, TITR
Cl	IC, INAA, SPEC, TITR
Mg	FAAS, ICP-AES, ICP-MS, IDMS, INAA
Mn	FAAS, ICP-AES, INAA
N	CATH, IDMS, SPEC, TITR, VOL
P	ICP-AES, RNAA, RREX, SPEC
S	IC, ICP-AES, IDMS, SPEC, TITR, IDMS
Zn	FAAS, ICP-AES, INAA

TABLE 6.17

CERTIFIED CONTENTS OF Cl, N, P AND S IN CRM 100, AND OF Al, Ca, Cl, Mg, Mn, N, P, S AND Zn IN CRM 101

	Certified value (g kg ⁻¹)	Uncertainty (g kg ⁻¹)	Number of accepted sets of results
CRM 100			
Cl	1.49	0.06	11
N	26.29	0.25	9
P	1.55	0.04	12
S	2.69	0.04	13
CRM 101			
Al	0.173	0.005	8
Ca	4.28	0.08	19
Cl	0.688	0.023	11
Mg	0.619	0.009	12
Mn	0.914	0.011	11
N	18.88	0.18	11
P	1.68	0.04	12
S	1.70	0.04	13
Zn	0.0353	0.0022	5

6.6.4. Participating laboratories

The sample collection and preparation has been carried out by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt in Münster (Germany), the Landesanstalt für Ökologie in Recklinghausen (Germany) and the EC Joint Research Centre in Ispra (Italy); the homogeneity study has been performed by the Department of Analytical Chemistry of the Complutense University in Madrid (Spain) and the Landwirtschaftliche Untersuchungs- und Forschungsanstalt in Münster (Germany); the stability study was carried out by the CNRS, Service Central d'Analyse in Vernaison (France).

The following laboratories participated in the certification campaign: An Forais Taluntais, Wexford (Ireland); Energieonderzoek Centrum Nederland, Petten (The Netherlands); GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Ökologische Chemie, Oberschleißheim (Germany); Institute for Reference Materials and Measurements, Geel (Belgium); Institut National de Recherches Agronomiques, Laboratoire Sols et Nutrition, Seichamps (France); Institut National de Recherches Agronomiques, Laboratoire d'Etude de la Pollution Atmosphérique, Seichamps (France); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Laboratório Nacional de Engenharia e Tecnologia Industrial, Lisbon (Portugal); Landesanstalt für Ökologie, Recklinghausen (Germany); NCR Demokritos, Agia Paraskevi, Attikis (Greece); Novo Industri A/S, Microanalytical Department, Bagsvaerd (Denmark); Queen's University, Department of Chemistry, Belfast (United Kingdom); Risø National

TABLE 6.18

COMPARISON OF METHODS FOR Ca, Cl, P, Mg, Mn AND S IN BEECH LEAVES CRM 100 AND SPRUCE NEEDLES CRM 101

Element	Techn. of final determination	CV (%) Between Means of lab. with the same technique	CV (%) Between means of diff. techniques
Chloride	INAA	3.2	0.9
	IC	4.9	
Phosphorus	ICP-AES	3.4	0.7
	SPEC	3.0	
Sulphur	ICP-AES	5.0	1.4
	IC	3.8	
	TITR	4.1	
Calcium	INAA	2.6	2.5
	ICP-AES	3.8	
	FAAS	2.6	
Magnesium	ICP-AES	2.1	0.9
	FAAS	2.8	
Manganese	INAA	2.4	0.2
	ICP-AES	2.1	
	FAAS	1.4	

Laboratory, Roskilde (Denmark); Service Central d'Analyse, CNRS, Vernaison (France); The Institute for Terrestrial Ecology, Cumbria (United Kingdom); Toegepast Natuurwetenschappelijk Onderzoek, Zeist (The Netherlands); Universidad de Córdoba, Departamento de Química Analítica, Córdoba (Spain); Universidad Complutense, Departamento de Química Analítica, Madrid (Spain); Universidad de Oviedo, Departamento de Química Física y Analítica, Oviedo (Spain); Università degli Studi di Pavia, Centro de Radiochimica, Pavia (Italy); Università La Sapienza, Dipartimento di Chimica, Rome (Italy); Universität Ulm, Sektion Analytik und Höchstreinigung, Ulm (Germany); University of Plymouth, Department of Environmental Sciences, Plymouth (United Kingdom).

6.7. TRACE ELEMENTS IN LICHEN

6.7.1. Introduction

Lichens are currently monitored to aid the control of air pollution and to follow changes in pollution patterns [20,21]. Lichens are found almost everywhere and accumulate trace elements from the atmosphere; therefore they are often used as a practical means for biomonitoring pollution [22,23]. The collection and analysis of

lichen material is much easier and cheaper than the use of air-filters; furthermore, the lichen monitoring produces data over wide geographical areas. The quality of lichen analysis is influenced by specific matrix effects that are not matched by the matrix of existing plant reference materials.

About 2000 papers involving lichen analysis have been published in recent years, and reveal high variability of data which may not only reflect different distribution pollution patterns but may also involve analytical errors; furthermore, lichen analyses are often performed for governmental bodies or industries which require to implement quality control (QC) by demonstrating accurate measurements. A rough estimate has shown that more than 5000 lichen analyses are performed every year within the European Community; this means that consequent economic losses are to be expected if the quality assurance (QA) of measurements performed is not verified. In order to improve and control the quality of trace element determinations of lichens, the BCR has hence launched a project the first step of which was intended to detect and remove the main sources of error likely to occur in lichen analysis through an interlaboratory study [24]; the results obtained in this exercise demonstrated that a certification campaign could be contemplated and a candidate reference material of lichen (CRM 482) was prepared for this purpose. This section presents the results of this certification for a range of trace elements.

6.7.2. Interlaboratory study

Two lichen samples, containing low and high trace element contents, have been prepared and distributed to 32 laboratories from 9 EC and 2 EFTA States. The interlaboratory study focused on the following elements: Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb and Zn. The results of the technical discussions are published elsewhere [24]. The evaluation could allow to detect sources of errors and to give recommendations to certifying laboratories prior to the certification. In addition, clear requirements for the preparation of the lichen candidate CRM were expressed, in particular washing was to be avoided as this procedure removes water-soluble and exchangeable ions such as e.g. K. The participants recommended that the lichen material be dried at room temperature and that all adhering particles be manually removed.

6.7.3. Production of the candidate CRM

The epiphytic lichen species *Pseudevernia furfuracea* was identified as a good candidate for a possible certified reference material. Some 40 kg of lichen was collected manually on pine trees located in the area of Axalp (Switzerland). The material was air-dried and coarse particles were removed manually. Following this pretreatment, the sample was stored in polyethylene bags that were transported at the Joint Research Centre of Ispra (Italy). The material was oven-dried at 105°C for at least 7 h to achieve a moisture content of about 8%. At this stage, the material appeared rather brittle while oven-warm but the fibres became rapidly elastic while picking-up moisture from the ambient air. The material was ground in a hammer-mill equipped with a 1 mm internal chamber sieve. The fraction <125 µm was collected in a drum flushed at regular intervals with

argon. The fraction $>125\ \mu\text{m}$ was ground again, using a $500\ \mu\text{m}$ chamber sieve and the resulting fraction $<125\ \mu\text{m}$ was added to the drum. The resulting material was homogenised for 14 days under dry argon. During this operation, long fibres aggregated and formed tiny balls with size ranging from some hundred μm to some mm; these aggregates were removed by sieving the material again at $125\ \mu\text{m}$ mesh. The fraction $>125\ \mu\text{m}$ was discarded and the sieved material was further homogenised for another two weeks. The material was stored in brown glass bottles with polyethylene inserts and plastic screw caps, each containing ca. 15 g of powder.

The homogeneity was verified by the determination of Al, As, Cd, Cr, Cu, Hg, Mo, Ni, Pb and Zn on intakes of 250 mg (200 mg for Hg). The determinations were performed by ICP-MS (As, Cd, Mo, Pb), ICP-AES (Al, Cu, Zn), ETAAS (Cr, Ni) and CVAAS (Hg), after HNO_3/HF digestion at 100°C for 14 h and 150°C for 2 h. Chlorine interference in As determination by ICP-MS was avoided by ion-exchange chromatography prior to ICP-MS injection. The results showed that the material is suitable for use as a CRM and is homogeneous at least at an analytical portion of 250 mg and above for the certified elements [25].

Bottles were kept at, respectively, -20°C , $+20^\circ\text{C}$ and $+40^\circ\text{C}$ over a period of 12 months and the elements considered for the homogeneity study were determined at regular intervals during the storage period. Besides the slight variations attributed to measurement uncertainties, no instability could be demonstrated. The material was stored at ambient temperature.

6.7.4. Certification

Eleven laboratories from nine countries participated in the certification (see section 6.7.5). Table 6.19 summaries the different techniques of final determination for the different elements as used by these laboratories. The pretreatment techniques were digestion with combination of acids in a pressurised or atmospheric mode, programmed

TABLE 6.19

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN LICHEN CRM 482

Element	Techniques used in certification
Al	DCP-AES, ETAAS, ICP-AES, ICP-MS, INAA
As	HAAS, ICP-MS, INAA, RNAA
Cd	DPASV, ETAAS, ICP-AES, ICP-MS, IDMS
Cr	ETAAS, IDMS, INAA, RNAA
Cu	DCP-AES, DPASV, ICP-AES, ICP-MS, IDMS, INAA, RNAA
Hg	CVAAS, ICP-MS, ID-ICPMS, IDMS, INAA, RNAA
Ni	DPASV, DCP-AES, ETAAS, ICP-AES, ICP-MS, IDMS, INAA
Pb	DCP-AES, DPASV, ETAAS, ICP-AES, ICP-MS, ID-ICPMS, IDMS
Zn	DCP-AES, DPASV, ICP-AES, ICP-MS, IDMS, INAA

dry ashing, microwave digestion, and irradiation with thermal neutrons. A detailed description of these methods is given in the certification report [25].

The spread of results for arsenic indicated relatively high ICP-MS results in comparison to data obtained by RNAA. Effects of ArCl interference on the determination of ^{75}As (particularly with chlorine contents as high as 7000 mg kg^{-1}) obviously caused such systematic difference to occur. It was agreed that this risk of systematic error was not acceptable for certification and that the ICP-MS datasets should be withdrawn. Lab.03 used an ion-exchange treatment with a chromatographic column prior to ICP-MS injection to remove the high chlorine contents and the set of results was consequently accepted.

A particular care was also recommended for the digestion of the lichen material. A systematic comparison of three microwave-decomposition techniques (with quartz vessels) using HNO_3 at 170°C demonstrated that a high pressure (85 bar) and an addition of HF was necessary to obtain a complete recovery of As in this material. The use of a high pressure (85 bar) mode and a medium pressure (10 bar) mode without addition of HF led respectively to As contents 35% and 70% lower than the one found with the high pressure/HF method. It was suspected that an adsorption effect of arsenic on the surface of the quartz vessels could have occurred, which was avoided by HF addition. All other sets of data were accepted for certification.

Low results obtained by DPASV for cadmium were suspected to be due to an incomplete decomposition of the organic matter; in addition, losses of volatile fractions were assumed with the dry ashing technique used (450°C , open system, instead of high pressure digestion). The set was consequently withdrawn.

A systematic difference was observed between high chromium values obtained by NAA techniques and ICP-MS and ICP-AES data. It was suspected that in some cases the digestion method used was not sufficiently strong to decompose the matrix; the addition of HF after HNO_3 decomposition is a prerequisite. The main problem was, however, related to strong interferences occurring at mass 52 with argon. As already mentioned for arsenic, ICP-MS and ICP-AES were not considered to be suitable for certification and the different sets were consequently withdrawn; ETAAS is clearly more reliable for chromium determination in comparison to ICP-MS.

Higher standard deviations in ICP-MS were observed for copper in comparison to ICP-AES results; this difference was due to a dilution step necessary prior to ICP-MS measurement (hence increasing the uncertainty) which was not necessary in ICP-AES; this larger uncertainty was considered to be acceptable for certification and the ICP-MS results were therefore kept.

In the case of lead, effects of different calibration methods using ICP-MS were investigated by one participant who found comparable results using isotope dilution and standard additions ($(37.1 \pm 0.2) \text{ mg kg}^{-1}$ and $(37.5 \pm 0.3) \text{ mg kg}^{-1}$, respectively) whereas higher results were obtained using external calibration ($(39.9 \pm 0.3) \text{ mg kg}^{-1}$). The latter technique was not recommended for certification. Systematic effects related to sample dilution were also observed. Since the ID-ICPMS results overlapped with the set of another laboratory using ICP-MS, they were accepted for certification. Doubts were expressed on a possible incomplete digestion performed by dry ashing and addition

of HNO_3/HF , which could explain low results of one participant. Further investigations carried out after the meeting demonstrated that higher values were obtained using another digestion method ($\text{HClO}_4/\text{HNO}_3$ at 170°C). Owing to this discrepancy, the participant withdrew all its sets of data.

For zinc, an incomplete digestion was suspected for two participants, possibly explaining low results. However, verification using an alternative method was carried by one of the two laboratories, which did not find significantly different results. The two sets of were therefore accepted for certification.

The certified values are given in Table 6.20. Indicative values were also given as described elsewhere [25,26].

The comparison of methods for some elements showed that no particular bias could be attributed to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 6.21).

Table 6.22 summarises the results of the certification (CVs of the means of laboratory means) in comparison with the results obtained in the intercomparison [24]. As shown in the table, the CVs obtained in the certification decreased drastically which illustrate that a considerable improvement could be achieved.

6.7.5. Participating laboratories

The sample collection and preparation has been carried out by the Arbeitsgemeinschaft für Bioindikation in Berne (Switzerland) and the Environment Institute of the EC Joint Research Centre of Ispra (Italy); the homogeneity and stability studies have been performed by LONZA A.G. in Visp (Switzerland).

The following laboratories participated in the certification campaign: Department of Chemistry of the Acadia University, Wolfville (Canada); Energieonderzoek Centrum Nederland, Petten (The Netherlands); GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Ökologische Chemie, Oberschleißheim (Germany); Istituto Superiore di Sanità, Rome (Italy); LONZA A.G., Visp (Switzerland); Risø National

TABLE 6.20

CERTIFIED CONTENTS OF TRACE ELEMENTS IN LICHEN 482

	Certified value (mg kg^{-1})	Uncertainty (mg kg^{-1})	Number of accepted sets of results
Al	1103	24	9
As	0.85	0.07	6
Cd	0.56	0.02	8
Cr	4.12	0.15	7
Cu	7.03	0.19	10
Hg	0.48	0.02	8
Ni	2.47	0.07	8
Pb	40.9	1.4	10
Zn	100.6	2.2	13

TABLE 6.21

COMPARISON OF METHODS FOR Al, As, Cr, Cu, Hg AND Zn IN LICHEN CRM 482

Element	Techn. of final determination	CV (%) Between means of lab. with the same technique	nr of sets of results	CV (%) Between means of diff. techniques
Aluminium	INAA	2.8	2	2.3
	ICP-AES	2.2	4	
Arsenic	INAA	5.4	2	9.2
	RNAA	7.1	2	
Chromium	INAA	2.2	2	2.2
	RNAA	4.7	3	
Copper	ICP-AES	4.2	3	0.7
	ICP-MS	2.4	2	
Mercury	ICP-MS	3.4	3	1.2
	CVAAS	0.3	2	
Zinc	INAA	2.8	2	3.8
	ICP-MS	1.8	3	
	ICP-AES	3.3	4	

TABLE 6.22

COMPARISON OF THE CERTIFICATION RESULTS WITH THE RESULTS OF THE INTERLABORATORY STUDY.

The results listed in this table correspond to the cvs of the means of laboratory means after technical scrutiny. tp24 and tp25 are the samples which were used in the intercomparison (adapted from [24]).

Element	CVs between labs. TP24*	CVs between labs. TP25*	CVs between labs. CRM 482
Al	6.3	7.3	2.8
As	13.1	13.1	7.8
Cd	24.1	23.8	2.6
Cr	19.1	14.0	3.9
Cu	13.3	11.7	3.6
Hg	18.2	15.5	3.1
Ni	18.8	25.1	3.1
Pb	20.0	7.6	4.5
Zn	13.1	10.2	3.6

Laboratory, Roskilde (Denmark); Department of Chemistry of the Saint Mary's University, Halifax (Canada); Service Central d'Analyse, CNRS, Vernaison (France); Universidad de Oviedo, Departamento de Química Analítica, Oviedo (Spain); Università

degli Studi di Pavia, Centro di Radiochimica, Pavia (Italy); Universiteit Gent, Instituut voor Nucleaire Wetenschappen, Ghent (Belgium).

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Chapter 7

CRMs for biological material analysis

7.1. TRACE ELEMENTS IN FISH

7.1.1. Introduction

Fish forms an important part of the human diet in particular in some countries. Fish is also used as an indicator of pollution of the marine environment. It is therefore widely analysed for nutritional studies, for assessing human exposure to toxic elements and to survey the marine environment.

7.1.2. Production of the candidate CRM

About 200 kg of cod fish were collected in the Southern part of the North Sea. The fish was filleted and all visible bones, skin, parasites and blood clots were removed. 75 kg of clean fillets were finally obtained and cut into pieces, packed in plastic bags in units of 1 kg and frozen. The whole procedure was done using titanium knives and taking all necessary precautions to prevent contamination or biological degradation.

The material was first crushed under constant cooling with liquid nitrogen in a jaw crusher of which the parts in contact with the fish were made of PTFE or similar material. Subsequent grinding was done in a teflon ball mill under liquid nitrogen. The material was freeze-dried after grinding to obtain a moisture content of ca. 1% (loss of mass of approx. 80%) and was bottled under a dry air atmosphere; 1200 bottles were filled each with 15 g of the freeze-dried material. This work is described in detail elsewhere [1,2].

The homogeneity was verified by the determination of Hg, K, Na, P, Se and Zn on intakes of 100 mg and 200 mg. In addition, a micro-homogeneity study was performed for Cd, Hg, Fe, Pb and Zn [4]. The samples were digested by pressurised ashing in quartz vessels at 170°C for 8 h with HNO₃. Analyses were performed by ICP (K, Na, P and Zn), CVAAS (Hg) and HAAS (Se). The micro-homogeneity study was performed by solid sampling Zeeman atomic absorption spectrometry. For Pb, Cd, Fe and Zn, pyrolytically-coated graphite was used for the furnace tubes as well as for the L'vov platforms. For Hg a special nickel tube furnace was used. Calibration was carried out using CRMs. Analyses by solid sampling ZETAAS (micro-homogeneity study) at different stages in the production process indicated that heterogeneities existing in the starting material were gradually eliminated in the subsequent production stages and that no external contamination occurred [1]. On the basis of the results, it was concluded that the material was homogeneous at least at a level of 100 mg and above. For selenium, an inhomogeneity was suspected at a level of 100 mg; however, the material was considered to be homogeneous at a level of 200 mg and above for this element [1]. The

results of the micro-homogeneity study demonstrated that the material was homogeneous at a level of 12 mg and above for Pb, 21 mg and above for Zn, 24 mg and above for Cd, 70 mg and above for Fe, and 400 mg and above for Hg.

Two sets of bottles were kept at +6°C and +20°C during a period of 12 months and As, Hg and Se were determined to assess the possible losses of volatile compounds. Samples were digested by high-pressure ashing with a mixture of HNO₃, HCl and H₂SO₄ at 320°C for 2 h. The final determination methods were HAAS (As, Se) and CVAAS (Hg). No instability could be demonstrated.

7.1.3. Certification

Table 7.1 summarises the different techniques of final determination used; pretreatment techniques were based on digestion with combination of acids in pressurised or atmospheric mode, programmed dry ashing, combustion and irradiation with thermal neutrons. A detailed description of the methods is given in the certification report [1].

For manganese, the FAAS technique was considered not to be sensitive enough for the certification of Mn in this material.

In the case of mercury, digestion for a few minutes was suspected not to be sufficient for a complete digestion of the organic matrix owing to high levels of methyl mercury (ca. 76% of the total Hg). In addition, losses of Hg were suspected with open digestion systems. Finally, the calibration by standard additions was found to be of paramount importance to avoid discrepancies: as an example, lab.15 detected (0.467 ± 0.014) mg kg⁻¹ of Hg using a calibration graph (external calibration) whereas a concentration of (0.533 ± 0.014) mg kg⁻¹ of Hg was found by using standard addition procedures. The highest levels were confirmed by solid sampling ZETAAS.

Care must be taken that the whole sample is digested. Pressurised digestions with oxidising acids were found to be suitable. Special care should be taken when using digestion for Hg analysis; it was indeed suspected that this method was not sufficient

TABLE 7.1

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN COD MUSCLE CRM 422

Elements	Techniques of final determination
As	HICP, HGAAS, ICP-AES, ICPMS, INAA, RNAA, ZETAAS
Cd	DPASV, ETAAS, IDMS, RNAA, SS-ZETAAS, SWASV, ZETAAS
Cu	DCP-AES, DPASV, ETAAS, FAAS, ICP-AES, IDMS, RNAA, ZETAAS
Fe	DCP-AES, ICP-AES, IDMS, INAA, ZETAAS
Hg	CVAAS, CVAFS, HICP, ICPMS, ID-ICPMS
I	INAA, RNAA
Mn	DCP-AES, ETAAS, ICP-AES, INAA, RNAA, ZETAAS
Pb	ICPMS, ID-ICPMS, IDMS, ZETAAS
Se	FLUO, HGAAS, HICP, INAA, RNAA, ZETAAS
Zn	DCP-AES, DPASV, FAAS, ICP-AES, ICPMS, IDMS, INAA, SS-ZETAAS

to achieve a complete digestion of organic mercury, which represents ca. 75% of the Hg present in the matrix.

An independent study was performed to study the limitations of microwave digestion for the analysis of this material [3].

An open digestion system was used and three different programmes were tested which are summarised in the Table 7.2.

The values obtained for some elements are presented in the Table 7.3.

As shown in Table 7.3, the results obtained for As, Cu, Mn and Zn are in good agreement with the certified values. In the case of As, determinations by HPLC/ICPMS have allowed to determine the respective amounts of As-species found after each treatment which were as follows:

Programme I: 84% of Asbetaine, 7% of As(III) and 9% of As(V)

Programme II: 80 to 90% of Asbetaine, 10 to 20% of As(V)

Programme III: 100% of As(V)

In the two first cases, the hydride generation method could not be used due to low recoveries owing to the fact that Asbetaine does not generate hydride. However, the HICP method gave good recovery when using the programme III (see Table 7.3) as all the arsenic was in the form As(V). The low values observed for Hg confirmed the discussions of the technical meeting. It was concluded that the microwave digestion methods have to be carefully verified for the determination of Hg in fish tissue due to the high content of methyl-mercury.

The certified values are listed in Table 7.4. Indicative values are given in the certification report [1].

The comparison of methods for some elements showed that no particular bias could be attributed to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 7.5)

7.1.4. Participating laboratories

The material was prepared by the Institute for Reference Materials and Measurements in Geel (Belgium); the homogeneity and stability studies were carried out by the GSF-Forschungszentrum für Umwelt und Gesundheit in Oberschleißheim (Germany).

The following laboratories participated in the certification: Biologische Anstalt Helgoland, Hamburg (Germany); Danish Isotope Centre, Copenhagen (Denmark); ECN Energieonderzoek centrum Nederland, Petten (The Netherlands); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); IFREMÉR, Nantes (France); Istituto Superiore di Sanità, Roma (Italy); Institut für Spektrochemie und angewandte Spektroskopie, Dortmund (Germany); Institute for Reference Materials and Measurements, Geel (Belgium); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); I.R.I./TU Delft, Delft (The Netherlands); Labor für Spurenanalytik, Bonn (Germany); National Food Agency, Søborg (Denmark); National Food Administration, Uppsala (Sweden); Presidio Multizonale di Prevenzione, Sezione Chimica, Venezia (Italy); Risø National Laboratory, Roskilde (Denmark), R.I.V.M., Bilthoven (The Netherlands); Service Central d'Analyse, CNRS, Vernaison (France); TNO-CIVO, Ijmuiden (The Netherlands); Universidad Complutense, Facultad

TABLE 7.2

MICRO-WAVE DIGESTION PROGRAMMES USED FOR ANALYSING CRM 422

PROGRAMME I			PROGRAMME II			PROGRAMME III		
Reagents	Time (min)	Power (W)	Reagents	Time (min)	Power (W)	Reagents	Time (min)	Power
10 mL HNO ₃	5	10	7 mL HCL + 3 mL HNO ₃	5	40	5 mL HNO ₃ + 2 mL H ₂ SO ₄	5	20
	10	30		10	50		10	40
	10	60		10	54		10	100
10 mL HNO ₃	10	60	1 mL H ₂ O ₂	5	40	5 mL HNO ₃	10	100
2 mL H ₂ O ₂	5	60	5 mL H ₂ O	5	50	1 mL H ₂ O ₂	5	100
5 mL H ₂ O	5	50				1 mL H ₂ O ₂	5	100
						5 mL H ₂ O	5	80

TABLE 7.3

RESULTS OF THE MICRO-WAVE DIGESTION TESTS FOR CRM 422

ELTS	PROGRAMME I		PROGRAMME II	PROGRAMME III		CERTIFIED VALUE
	ICP	ICPMS	ICP	ICP*	ICPMS	
As	20.3 ± 1.2		24.33 ± 0.35	21.36 ± 0.34	19.54 ± 1.2	21.1 ± 0.7
Cu		1.07 ± 0.15				1.05 ± 0.11
Hg		0.440 ± 0.05			0.488 ± 0.05	0.564 ± 0.015
Mn	0.514 ± 0.03	0.549 ± 0.05		0.521 ± 0.032		0.543 ± 0.042
Zn	19.5 ± 0.5		19.89 ± 0.20		19.6 ± 0.9	

* for As: HICP

TABLE 7.4

CERTIFIED CONTENTS OF As, Cd, Cu, Fe, Hg, I, Mn, Pb, Se AND Zn IN CRM 422

	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
As	21.1	0.5
Cd	0.017	0.002
Cu	1.05	0.07
Fe	5.46	0.30
Hg	0.559	0.016
I	4.95	0.49
Mn	0.543	0.028
Pb	0.085	0.015
Se	1.63	0.07
Zn	19.6	0.5

TABLE 7.5

COMPARISON OF METHODS FOR Cu, Mn, Se AND Zn IN CRM 422 (COD MUSCLE)

Element	Techn. of final determination	CV % between means of lab. with the same technique	Nr of sets or results	CV % between means of diff. techniques
Cu	ICPAES	7.8	4	6.7
	ZETAAS	14.5	4	
Mn	ICPAES	6.9	3	5.2
	ZETAAS	6.0	3	
Se	HICP	1.6	3	4.3
	HAAS	2.3	4	
Zn	ICPAES	7.5	4	2.6
	FAAS	1.4	6	
	INAA	3.7	3	

de Quimica, Madrid (Spain); Universidad de Oviedo, Facultad de Quimica, Oviedo (Spain); Università degli Studi di Pavia, Centro di Radichimica Generale, Pavia (Italy); Universitaire Instelling Antwerpen, Departement Scheikunde, Wilrijk (Belgium).

7.2. METHYL MERCURY IN FISH

7.2.1. Introduction

Methyl mercury (MeHg) may be directly released in the environment (e.g. in Mina-Mata, Japan, from poly-vinyl wastes) or originate from the biomethylation of

inorganic mercury in biological tissues [4,5]. This highly toxic compound accumulates in the food chain and affects biota and humans. Therefore MeHg is to be determined accurately in environmental matrices and food. Legislation on MeHg in food is contemplated in some countries to be preferred above legislation on total Hg; so far such legislation was impossible because of the lack of reliability and accuracy of existing methods. Hence, there was an urgent need for validating analytical methods for MeHg determination. Many methods have been described but a systematic collaborative investigation of their performance for the analysis of fish and mussel tissues has shown that, although the results were considered to be acceptable by the participants [6], the coefficient of variation between laboratories was in the 20–25% range, which is not sufficient for allowing an accurate comparison of data to be made. There are various ways to determine MeHg, the majority of those consisting of an extraction, a separation and an identification/quantification step. Extraction is often performed with a lipophilic solvent or the sample is destroyed in an alkaline solution followed by a selective reduction (e.g. with SnCl_2). The separation and identification can be carried out by gas chromatography, ion exchange or high-pressure liquid chromatography. Techniques such as cold vapour atomic absorption spectrometry (after selective separation), electron capture detection and mass spectrometry are generally used for the detection and quantification. Radiochemical methods and head-space gas chromatography are also applied. The complexity of the methods and the multiplicity of analytical steps are the reasons why errors are easily made.

In view of the urgent need for the improvement of the quality of the analyses, a project for MeHg has been discussed and designed with a group of experts in the frame of the BCR-Programme. The programme of work was set up in the form of an improvement scheme (see Chapter 12). In particular, the various steps of the analytical methods were studied individually by each of the participants: e.g. extraction, clean-up and separation. This improvement scheme is fully described in Chapter 12; the present section gives a summary of this certification of tuna fish, which followed the interlaboratory studies.

7.2.2. Production of the tuna fish reference materials

The two candidate reference materials were collected in the Adriatic Sea; they were produced from tuna fishes that were rejected from the normal trade because their total mercury content exceeded 0.8 g kg^{-1} . 302 kg and 322 kg of tuna fish, respectively for CRM 463 and CRM 464, were sliced, frozen (-25°C) and transported to Ecoconsult in Gavirate (Italy). The dorsal fish muscles of each material were minced using a Quick Mill 2300 mincer with tungsten carbide blades. After mincing, the material obtained was stored frozen in high-density polyethylene containers. The material was then freeze-dried until reaching a moisture mass fraction below 2.5%. The resulting material (ca. 36 kg of each candidate CRM) was immediately frozen.

The freeze-dried materials were sent to the Joint Research Centre of Ispra where they were ground using a mill equipped with zirconium dioxide balls. The ground materials were sieved using a vibrating stainless steel sieve. The fractions with particles larger than $125 \mu\text{m}$ were discarded and the remaining materials were stored in polyethylene

boxes in an argon atmosphere. The two materials were then homogenised in a mixing drum for 16 days and bottled in brown borosilicate glass bottles. A total of 1000 bottles each containing ca. 15 g of material was produced for each candidate reference material. Both materials were stored at a temperature of 4°C.

The between bottle homogeneity was verified by the determination of total and methyl mercury on sample intakes of 0.2 g. For the determination of total mercury, the samples were mineralised by digestion using nitric acid. The final determination was performed by CVAAS. Methyl mercury was determined by CGC/ECD after extraction of 0.2 g fish powder in toluene, back extraction with a cysteine acetate solution and further extraction with toluene. Calibrations were performed by standard additions. The study showed that the two materials are homogeneous at an analytical portion of 0.2 g and above for total and methyl mercury [7].

Bottles were kept at respectively -20°C, +20°C and +40°C over a period of 12 months and total and methyl mercury were determined at regular intervals during the storage period. No instability could be demonstrated [7].

7.2.3. Certification

The techniques used in the certification involved solvent extraction (MeHg) or acid digestion, either pressurised, under reflux or -based (total Hg), derivatisation (e.g. NaBH₄), separation by capillary gas chromatography (CGC) and various methods of final detection (e.g. AAS, ECD, MIP). Table 7.6 gives an account of the techniques used by each laboratory in the certification for methylmercury; in the case of total mercury, the final determination techniques were CVAAS, CVAFS, ICPMS and RNAA. More details on the method description can be found in the certification report [7], including also important precautions taken in the certification to avoid sources of error.

The verification of the concentrations of the calibration solutions was considered to be a important aspect to achieve traceability. A pure methyl mercury chloride compound was prepared for the certification, the purity of the calibrant of which was verified by C, H, Hg and Cl elemental analysis and was found to be higher than 99.8%. This compound was distributed to the participants in the certification both for calibration purposes and as a mean of verification of the calibrant used in their laboratory.

For total mercury, the use of polyethylene vials in INAA was suspected to have lead to evaporation losses during the irradiation owing to the high amount of volatile methyl

TABLE 7.6

SUMMARY OF TECHNIQUES USED IN THE CERTIFICATION OF METHYLMERCURY IN CRMS 463 AND 464 (TUNA FISH)

Compound	Techniques of separation and final determination
MeHg	CVAAS after cysteine extraction, IE/UV/AAS, GC/AFS, CGC/ECD, CGC/FTIR, CGC/MIP, GLC/CVAFS

mercury present in the tuna fish materials. The laboratory re-analysed the samples using quartz ampoules and found better results, which were accepted.

To check whether the apparently lower results of Lab. 13 could be ascribed to the short duration of their microwave digestion procedure (3 h at 70°C with HNO₃ in an open system), the laboratory compared the 3 h digestion procedure with a longer digestion (24 h) and could not observe any difference. The results were accepted.

In the case of methylmercury, The results of Lab. 10 were on the low side for the CRM 463. The recovery of the distillation procedure had been verified by a spike (distillation stopped on the basis of the distilled spike solution). Usually, the recovery ranged from 90 to 95% but in the case of CRM 463 it varied between 68 and 95% which was due to a too low MeHg spike content compared to the methyl mercury present in the sample. This procedure was not considered to be acceptable and the results were therefore rejected.

The results of Lab. 15 were found to be low which was due to the fact that the calibrants were added after the digestion step (with HCl) which resulted in different treatment between calibrants and samples and possible different extraction efficiencies. The laboratory withdrew its results.

The certified values are given in the Table 7.7.

7.2.4. Participating laboratories

The preparation of the tuna fish samples was carried out at the Joint Research Centre (Ispra, Italy). The following laboratories participated in the interlaboratory programme: Bundesforschungsanstalt für Fischerei, Hamburg (Germany); Danish Isotope Centre, Copenhagen (Denmark); IFREMER, Nantes (France); Institut Jozef Stefan, Ljubljana (Slovenia); Kernforschungsanlage, Jülich (Germany); De Montfort University, School of Applied Physical Sciences, Leicester (United Kingdom); MRC Toxicology Unit, Carshalton (United Kingdom); National Food Administration, Uppsala (Sweden); National Food Agency, Søborg (Denmark); Presidio Multizonale di Prevenzione, Laboratorio Chimico, La Spezia (Italy); Presidio Multizonale di Prevenzione, Sezione Chimica Ambientale, Venezia (Italy); RIKILT, Wageningen (The Netherlands); Swedish

TABLE 7.7

CERTIFIED CONTENTS OF TOTAL AND METHYL MERCURY IN CRMS 463 AND 464

Compound	Certified value	Uncertainty	Unit	p
CRM 463				
total Hg	2.85	0.16	mg kg ⁻¹ (as Hg)	8
MeHg	3.04	0.16	mg kg ⁻¹ (as MeHg)	11
CRM 464				
total Hg	5.24	0.10	mg kg ⁻¹ (as Hg)	8
MeHg	5.50	0.17	mg kg ⁻¹ (as MeHg)	12

Environmental Research Institute, Göteborg (Sweden); Universidad Complutense, Departamento de Química Analítica, Madrid (Spain); Universidad de Santiago de Compostella (Spain); Università di Genova (Italy); Vrije Universiteit Brussel, Lab. voor Anal. Scheikunde (Belgium); University of Umeå (Sweden).

The following participants provided data for total Hg in the certification campaign: Energieonderzoek Centrum Nederland, Petten (The Netherlands); FORCE Institute, Brøndby (Denmark); Service Central d'Analyse, CNRS, Vernaison (France); Universiteit Gent, I.N.W., Ghent (Belgium).

7.3. As-SPECIES IN TUNA FISH

7.3.1. Introduction

Arsenic is an ubiquitous element which occurs in the form of various chemical species in the environment. In biological tissues, the main species identified is arsenobetaine which is considered to be non toxic and present at more than 90% in fish tissues and does not exceed 50% in molluss [8]. Other species such as arsenocholine, tetramethylarsonium ion, trimethylarsenoxide, dimethylarsinic acid and arsenosugars have also been identified [9].

Several intercomparisons have been organised from 1989 to 1995 to improve the state of the art of As-speciation analysis [10]. The number of exercises needed to enable the certification of some arsenic species in tuna fish and solutions [11] illustrated the high degree of difficulty of this type of analysis. Interlaboratory exercises organised by BCR followed a stepwise approach consisting of six exercises of increasing difficulty, namely solutions of six pure arsenic species (arsenite As(III), arsenate As(V), monomethylarsonic and dimethylarsinic acids MMA and DMA, arsenobetaine and arsenocholine), solutions containing a mixture of the six arsenic species, solutions containing the six arsenic species together with interfering cations and anions, fish and mussel raw extracts, fish and mussel cleaned extracts, and shark and mussel powders. The certification of total arsenic, dimethylarsinic acid (DMA) and arsenobetaine in tuna fish (CRM 627) was completed in 1996, along with the certification of arsenobetaine in solution (CRM 626); this section describes the certification of As-species in tuna fish.

7.3.2. Production of the materials

The tuna fish material (CRM 627) was prepared by the Joint Research Centre, Environment Institute, of Ispra (Italy) whereas the arsenobetaine calibrant was prepared by the Laboratoire de Chimie Analytique et Minérale in Strasbourg (France) [12].

The tuna fish material was obtained from the city of Venice (fish removed from the fish market, owing to its too high content of mercury). The fish were caught in the Messina strait, kept frozen for approximately four weeks, dissected and the dorsal muscles were taken; these were minced and freeze-dried, ground in a zirconium dioxide mill, passed over a 125 µm sieve and the fraction >125 µm was discarded.

The fraction less than 125 µm was collected and homogenised in a special PVC mixing drum filled with dry argon. The homogenised powder was subsampled and tested for bulk homogeneity by XRF, choosing a number of minor and trace key elements. The material was filled in brown glass bottles with plastic inserts and screw caps. The bottles were primarily flushed with dry nitrogen and stabilised by irradiation (^{60}Co) to avoid microbiological decay.

The homogeneity was verified by repeated determinations of total As, arsenobetaine and DMA. The total As content was determined by HG-QFAAS after assisted digestion whereas DMA was determined by HPLC-ICPMS. No inhomogeneity was suspected at a level of 0.3 g for total As and 1 g for As-species, and the material was considered to be suitable for use as a CRM.

The stability of arsenobetaine and DMA in the tuna fish was tested over a period of 9 months at -20°C , $+20^{\circ}\text{C}$ and $+40^{\circ}\text{C}$. In addition, the stability of the material and of the raw extract was also verified by a qualitative control of the chromatograms; no unexpected peaks containing arsenic were detected. The results showed that no instability could be demonstrated for both arsenobetaine and DMA at $+20^{\circ}\text{C}$ and $+40^{\circ}\text{C}$ [12].

7.3.3. Certification

Several methods have been developed for the determination of arsenic species, involving different extraction, derivatisation, separation and detection steps [13]; these include hyphenated techniques based on liquid chromatography coupled to detectors such as ICP-MS or ICP-AES, and hydride generation in line with QFAAS and UV degradation followed by ICP-AES detection. The techniques that were selected in the certification campaign are listed in Table 7.8.

Since one of the most difficult features faced in the project was the lack of commercially available calibrant, a set of calibrants has been especially prepared for the purpose of the interlaboratory studies and certification. A full description of the synthesis is given elsewhere [12].

Total arsenic as well as arsenobetaine and dimethylarsinic acid had to be quantified. Inorganic species (As(III) and As(V)), as well as arsenocholine and monomethylarsonic acid were not detected by the various methods described in Table 7.8.

Almost all the participants have used an extraction with water/methanol 1:1 (v/v) mixture with or without ultrasonic assistance. It has been verified that this process does

TABLE 7.8

SUMMARY OF TECHNIQUES USED IN THE CERTIFICATION OF TOTAL As, DMA AND As BETAINES IN CRMs 627 AND 628

Compounds	Techniques of final determination
Total As	EDXRF, HG-QFAAS, HG-ICP-AES, ICPMS
DMA and As-betaine	HG-GC-QFAAS, LC-UV-ICP-AES, LC-UV-HG-QFAAS, LC-ICPMS

not induce arsenobetaine, DMA, MMA, As(V) or arsenocholine degradation. The optimisation of the power of the ultrasonic bath is an important feature for achieving the best extraction yields; nevertheless, the bath must be refrigerated to avoid degradation of the compounds. Enzymatic digestion with trypsin is also possible but the activity of the enzyme must be strictly controlled before use to guarantee the reproducibility of the method.

One laboratory did a purification on a silica column, which is a source of possible losses and should be avoided. Filtration with C₁₈ proved to be efficient and did not lead to losses of arsenobetaine, DMA, MMA or As(V).

In term of mass balances, two laboratories found a concentration of total arsenic in the powder lower than the sum of the various arsenic species detected. In one case, this was due to an incomplete digestion of the solid and in the other case to a calibration error in the determination of arsenobetaine. Some participants found traces of arsenocholine, MMA or As(V) but the contents were too small to be quantified; consequently, only DMA, arsenobetaine and total As contents were certified.

The certified values were (51.5 ± 2.1) mmol kg⁻¹ for arsenobetaine (6 sets of results); (2.04 ± 0.27) mmol kg⁻¹ for dimethylarsinic acid (6 sets of results); and (4.8 ± 0.3) mg kg⁻¹ for total As (9 sets of results).

7.3.4. Participating laboratories

The overall coordination of the interlaboratory studies and certification campaign was managed jointly by the Laboratoire de Chimie Analytique et Minérale of the Université Louis Pasteur in Strasbourg (France) and the CNRS, Service Central d'Analyse in Vernaison (France). The Environment Institute of the Joint Research Centre of Ispra (Italy) prepared the candidate reference material of tuna fish. Arsenobetaine calibrant for the certification was synthesised and characterized in the Laboratoire de Chimie Analytique et Minérale in Strasbourg (France).

The following laboratories participated in all the interlaboratory studies: CNRS, Service Central d'Analyse, Vernaison (France); CNRS, Laboratoire de Chimie Analytique et Minérale, Strasbourg (France); IFREMER, Nantes (France); Institut Pasteur, Service Eaux-Environnement, Lille (France); Istituto Superiore di Sanità, Roma (Italy); National Food Agency, Søborg (Denmark); State Laboratory, Dublin (Ireland); Universidad de Barcelona, Departamento de Química Analítica, Barcelona (Spain); University of Plymouth, Department of Environmental Sciences, Plymouth (United Kingdom); University of Southampton, Department of Chemistry (United Kingdom).

7.4. CBs IN FISH OIL

7.4.1. Introduction

Chlorobiphenyls (CBs) are a class of 209 discrete chemical compounds in which between one to ten chlorine atoms are attached to the biphenyl nucleus. The commercial products are complex mixtures used widely in industry, including transformers, capacitors,

printing inks, paints, and paper industry. Their long-term chemical and physical stability and their disposal in the environment has caused a wide-spread contamination problem. Chlorobiphenyls do not readily degrade biologically; they are lipophilic and tend to accumulate in biota. These contaminants concentrate in most environmental compartments, in human and animal adipose tissue, milk, sediment, sludge and marine and freshwater biota. Of the potential 209 congeners, only about 120 have been detected in the environment. Since routine analysis of anything approaching this number of components in each sample would be prohibitive on a cost basis, a selection of congeners was made, using the following criteria: presence in industrial mixtures; occurrence in a wide number of environmental samples; toxicity of individual congeners; and current analytical state-of-the-art. CBs Nos 28, 52, 101, 138, 153 and 180 were selected because they occur in many environmental samples and they can be separated from most matrix interferences and co-eluting CBs; CB No 118 was selected on the basis of its toxicity. The certification of these CBs in fish oil reference materials was requested for quality control as an aid to control maximum allowable concentrations in fishery products, identification of CB pollution sources, and environmental time trend analysis. The fish oils are representative of those analysed in control laboratories and fishery institutes and the CB concentrations in the oils are of sufficiently wide range to be regarded as suitable for the control and optimisation of analytical methods used to determine CBs in lipophilic matrices [14].

7.4.2. Interlaboratory studies

Series of interlaboratory studies were carried out prior to certification in order to evaluate the performance of current analytical techniques [15]. The group of participating laboratories examined the repeatability and reproducibility of the final determination of CBs calibrant solutions. Once the detection systems were optimised, the reproducibility of the calibrant solutions was checked with independent primary calibrants.

An eel extract and a sewage sludge were prepared and half the extracts cleaned-up by a single laboratory. The second half of the extracts were cleaned-up by each laboratory using the different methods later employed in this certification project in an optimised form. Both sets of samples were analysed for the seven CB congeners to determine the level of variance amongst the clean-up procedures.

Finally, a series of laboratory reference materials were examined to determine the interlaboratory reproducibility. At each stage, recommendations were made for improvement in the analytical method and are briefly summarised below.

Calibrant solutions should be made from high purity materials and dissolved in iso-octane. In general, it is recommended that an internal standard is used which is pure, thermally and photochemically stable and which is not present in the environment (e.g. DCBEs). This should be added to the calibrant solutions and to the samples prior to injection. If the internal standard used is a synthesised CB that is not present in industrial PCB mixtures it may be added at the commencement of the analysis (prior to the clean-up procedure). There was no significant difference in the results obtained using different internal standards, but some interferences of tetrachloronaphthalene (TCN) was detected in the chromatogram of some samples.

The optimum conditions for capillary gas chromatography were as follows: (1) the recommended temperature for the splitless injector was between 25°–270°C and ambient temperature for the on-column injector. The initial oven temperature when using iso-octane as solvent should be 90–130°C for the on-column injector and 90–100°C for the splitless injector; (2) the programme rate used ranged from 3 K.min⁻¹ to 10 K.min⁻¹ and was adjusted for maximum resolution between CB 153 and CB 138; (3) there was no significant difference in the data obtained using a splitless injector and an on-column injector for both a series of calibrant solutions and an eel extract [15]; therefore, either type of injector may be used.

The linearity and the calibration of the detector were amongst the most critical parameters to be determined in the optimisation of the final detection. They were predetermined by injecting each CB over the mass range 0–500 pg and plotting the ratio of the response to mass against the mass injected. The linearity of the ECD was less dependable below 25–50 pg. The linear response of the detector should be determined with the calibration made with the proven linearity of the system. The calibration should be carried out using bracketing or multiple calibrants to produce a calibration curve. Calibration at one single concentration is not acceptable.

During this optimisation programme, samples of eel, fish oil, mussel and sewage sludge were prepared for intercomparison. Data from analysis of a crude eel extract and a refined (cleaned-up) sample were compared to test the variance associated with different methods of sample preparation. The clean-up methods included gel permeation chromatography, saponification, concentrated sulphuric acid treatment, alumina and silica-gel column chromatography. The differences in results of the within and between clean-up methods did not significantly influence the overall analytical variance after the appropriate optimisation.

The programme enabled to select the most appropriate capillary column in order to minimise chromatographic interferences; recommendations for the most suitable columns are given elsewhere [14,15].

7.4.3. Production of the materials

Samples of cod and mackerel were collected in the North Sea, frozen immediately following their capture and transported at –25°C; they were stored at this temperature until they were defrosted prior to the preparation of the fish oils.

The cod livers, totalling 37.5 kg, were carefully dissected and cooked in water (10 L) for 30 minutes. The upper layer of cod liver oil was centrifuged twice, once to remove the solid particles and a second time to remove the water. The oil (10 L) was mixed thoroughly and collected in a clean round bottomed flask under argon while it was still hot.

The mackerel (40 kg) was cooked whole in water (45 L) in three batches. The mixture of mackerel and water was pressed to remove the oil while the fish was still hot; the oil was separated and cleaned in the same way as the cod liver oil.

The oils were stabilised by addition of 0.02% (by mass) butyl-hydroxy-toluene (BHT) as an antioxidant. The fish oils were ampouled in brown glass vials, which were previously tested for CB contamination prior to filling with the fish oils. The ampoules were

rinsed twice with n-pentane and the concentrated extracts were analysed by gas chromatography. No detectable quantity of any CB was found. The ampoules were flushed with argon and filled with the fish oils. The mackerel oil was kept at 40°C to maintain a homogeneous mixture. Care was taken to prevent the oil from contaminating the neck of the vial, which was flame sealed under argon immediately after filling. About 1200 ampoules of each of the two fish oils were prepared (each containing ca. 2 g).

The homogeneity was verified by determining the seven CBs and the total lipid content in the two fish oils in 30 ampoules taken from the whole batch. Oil (1.6 g) from each ampoule was dissolved in pentane. The ampoules containing the mackerel oil were heated to 45°C before opening to dissolve any partially solidified fats which may have formed when the oil is stored in a cool place. Oil (0.2 g) was also taken from each ampoule to determine the fat content. For the determination of CB-homogeneity a portion of each fish oil equivalent to 240 mg of lipid was cleaned-up on an alumina column (15 g Al_2O_3 , 6% H_2O by mass) and eluted with 12.5 mL of 2,2,4-trimethyl pentane (iso-octane) to separate the chlorinated hydrocarbons from lipids, waxes and other polar co-extracted compounds. The eluate was concentrated on a rotary evaporator and the CBs were separated from other organochlorines on a silica gel column (1.5 g, SiO_2). Hexabromobenzene was added as an internal standard (0.08 mg L^{-1}); 2 μL were injected into a gas chromatograph and detection was carried out with an electron capture detector [14]. A slight discrepancy was observed between the between-ampoule CV and the CV of the method, which was rather attributed to the difficulty in the determination than to any inhomogeneity. [1] This was confirmed by the results obtained in the certification campaign; the increase of CV was particularly obvious at lower concentrations since non-reproducible factors of the chromatographic system become more significant at the lower signal levels (e.g. traces of septum debris in the injector, condition of the column etc.).

The stability of the fish oils was tested at -18°C, +20°C and +37°C over a period of 14 months. There was no significant difference associated with stability in the concentrations of CBs or the total lipid content in either fish oil stored at the three temperatures. This stability was expected as it is well known that CBs are very stable. The matrix stability was assured by the measures taken such as use of dark glass ampoules and sealing under argon. Storage at a temperature at ca. +20°C is acceptable for long-term stability; a higher temperature up to +37°C is unlikely to cause any measurable effect on the CB-content.

7.4.4. Certification

The method of analysis for the final determination of the seven CB congeners used by each of the participating laboratories was based on capillary gas chromatography with electron capture detection. In addition, mass spectrometry was used for compound identification and confirmation, but not for quantification. Each laboratory used their own proven procedures for the sample preparation, clean-up, method of injection, choice of carrier gas and chromatographic condition. The fish oils were dissolved in an appropriate solvent and analysed without any preliminary extraction from the matrix.

The solutions of the fish oil were prepared immediately after opening the ampoule; the clean-up and the final determination were completed within the normal time scale for these analyses.

A set of calibrants containing the seven CB congeners was supplied to each participating laboratory in the form of pure, crystalline, certified materials from BCR (CRMs 291–298). Each laboratory was requested to prepare separate calibration solutions of the appropriate concentration in iso-octane, to determine the linearity of the electron capture detector and to calibrate the gas chromatograph prior to the analysis of the fish oils.

The choice of internal standards was left to the participants. However, a set of pure DCBEs (dichloro-benzyl-alkyl-ethers) was made available to each laboratory by the Freshwater Fisheries Laboratory for use with the calibrants provided and the samples.

A detailed description of the methods used is given in the certification report [14].

Results of participating laboratories were generally in good agreement, which enabled all seven CB congeners to be certified. The only sources of errors detected in two laboratory's sets of data were due to an insufficient separation of CB 52 from three accompanying peaks, and results found to be beyond the calibrated linear range of the detector for CB 153.

The certified values are given in Table 7.9. Confirmatory and indicative values are included in the certification report.

7.4.5. Participating laboratories

The preparation of the materials and the verification of their homogeneity and stability was carried out by the Netherlands Institute for Fishery Investigations (RIVO) in IJmuiden (The Netherlands). The following laboratories participated in the certification: Department of Agriculture and Fisheries for Scotland, Pitlochry (United Kingdom); Fisheries Research Centre, Amsterdam (The Netherlands); Free University of Amsterdam

TABLE 7.9

CERTIFIED CONTENTS OF CBs IN COD LIVER OIL CRM 349 AND MACKEREL OIL CRM 350

Compound (CB IUPAC Nr)	Cod liver oil (CRM 349) Certified value ± uncertainty ($\mu\text{g kg}^{-1}$)	Mackerel oil (CRM 350) Certified value ± uncertainty ($\mu\text{g kg}^{-1}$)
28	68 ± 7	22.5 ± 4.0
52	149 ± 20	62 ± 9
101	370 ± 17	165 ± 9
118	456 ± 31	143 ± 20
138	765 ± 45	274 ± 27
153	938 ± 40	318 ± 20
180	282 ± 22	73 ± 13

(The Netherlands); Institute of Marine Research, Bergen (Norway); Institut Français de Recherche pour l'Exploration de la Mer, Brest (France); Istituto Inquiritamento Atmosferico, Roma (Italy); Laboratoire Municipal de la Ville de Rouen (France); Milchwirtschaftliche Untersuchungs und Versuchsanstalt, Kempten (Germany); National Food Agency, Søborg (Denmark); National Swedish Environment Protection Board, Solna (Sweden); University of Ulm (Germany); Netherlands Institute for Fisheries Investigations (RIVO), IJmuiden (The Netherlands); State Institute for Quality Control of Agricultural Products (RIKILT), Wageningen (The Netherlands).

7.5. ORGANOCHLORINE PESTICIDES IN COD LIVER OIL

7.5.1. Introduction

Organochlorine pesticides (OCPs) such as DDT, chlordanes and their metabolites, hexacyclohexanes, the drins and chlorinated benzenes are chemically and physically stable compounds. Large amounts of these compounds have been released into the environment and subsequently have been found in all studied environmental compartments. Although the majority of these OCPs have been banned or are used in a restricted manner, their residues are still found in environmental matrices. This is a result of the physical and chemical persistence and the recycling of these compounds in the natural environment. Many of these compounds are of a lipophilic nature and have the ability to bioaccumulate through the food chain. The metabolites of these industrial chemicals result from a biological and environmental degradation. They are often more toxic and persistent than the parent compounds. It is therefore necessary to analyse these compounds in order to monitor their concentrations and effects. Data of good quality is required if they are to be assessed in terms of their environmental impact and in the formulation of environmental policy. A fish oil is a perfect matrix for monitoring of the marine environment for the persistence of OCPs in animals and a cod liver CRM was hence prepared for QC purposes [16].

7.5.2. Production of the candidate reference material

Samples of cod were taken from the North Sea and frozen immediately after capture and transported at -25°C . They were stored at this temperature until they were defrosted prior to the preparation of the oil material. The cod livers were carefully dissected and cooked in water for 30 min. The upper layer of cod liver oil was centrifuged twice, once to remove solid particles, and a second time to remove water. The oil was mixed and collected in a clean round-bottomed flask under argon while it was still hot.

The cod liver oil reference material was produced by mixing two different previously characterised cod liver oils. The mixing enabled to reach a medium level of OCP contamination, compared to usually analysed samples.

2.5 L of a highly contaminated cod liver oil (A) was mixed with 4.8 L of a relatively uncontaminated cod liver oil (B) in a 10 L reagent bottle by means of a mechanical stirrer. Prior to mixing, 200 mL of oil (B) was gently heated to 60°C to dissolve 2.5 g

Butylated Hydroxy Toluene (BHT) used as an antioxidant. The aliquot was cooled, returned to the bulk and mixed by stirring.

The material was again mixed prior to ampouling. The material was dispensed into cleaned amber glass ampoules using a calibrated dispensing pump and cleaned silicon rubber tubing. Each ampoule was filled with 5 g of material. Care was taken that no oil remained in the neck of the ampoule. The ampoules were sealed under argon. This process was carried out in batches of 40 ampoules. One ampoule out of each batch of 40 was randomly set aside for the homogeneity study. In total, 1200 ampoules of the material were prepared. The ampoules were then stored in a freezer at -20°C prior to analysis.

The between-ampoule and within-ampoule homogeneity of the material was verified by the determination of the chlorinated pesticides HCB, α -HCH, γ -HCH, p,p'-DDE, o,p'-DDD, o,p'-DDT, p,p'-DDD, p,p'-DDT, dieldrin, oxychlordane, α -chlordane, γ -chlordane and trans-nonachlor. β -HCH was not included in the homogeneity study as it coeluted with γ -chlordane in both samples and calibrants on the capillary column selected. Results for o,p'-DDE could not be used as a small non reproducible amount of o,p'-DDT degraded in the injector of the GC. This led to large CVs for o,p'-DDE but did not affect significantly the repeatability of the o,p'-DDT measurements. The ampoules were opened at room temperature and a sub-sample of ca. 0.2 g was removed, dissolved in 2 mL of n-hexane and then gently evaporated to 1 mL in a stream of clean air. This concentrate was then quantitatively transferred to the top of a column containing 6 g Al_2O_3 deactivated to 3% with distilled water and was eluted with 100 mL n-hexane. The total eluate was collected and its volume was reduced to 5 mL using a rotary evaporator before transferring quantitatively to a graduated test tube and reduction to 1 mL under a stream of clean air. The sample was then added to a 3 g Al_2O_3 column deactivated to 3% with distilled water and eluted with 25 mL n-hexane. The first fraction (fraction A1) of ca. 4 mL, containing CBs, HCB, p,p'-DDE was collected in a test tube while the second fraction containing the remaining OCPs (fraction B1) was collected in a 100 mL round bottomed flask and stored.

Fraction A1 was evaporated to 1 mL under clean air and quantitatively transferred to the top of a 3 g silica column deactivated to 5% with distilled water. The sample was eluted with 25 mL n-hexane. The first 6 mL were collected and evaporated to 0.5 mL under a stream of clean air. This fraction contained CBs, HCB and p,p'-DDE (fraction A2). The volume was made up to 10 mL with iso-octane, which was then evaporated to 1 mL. The remaining eluate of fraction A1 was added to the fraction B1 which was then evaporated using a rotary evaporator, transferred to iso-octane as above and evaporated to 1 mL in a stream of clean air (fraction B2) solution. 500 μL of an internal standard 2,4-dichlorobenzylalkyl ether (DCBE-6, DCBE-16) was added quantitatively to the fraction A2, which was subsequently evaporated to 500 μL . Fraction A2 was injected (0.5 μL) onto a Cpsil 8, 50 m fused-silica capillary GC column and fraction B2 was injected (0.5 μL) onto a Cpsil 19, 50 m fused-silica capillary GC column of which the GC conditions are given in the certification report [16]. There was no significant difference between the means of the between-ampoule homogeneity analysis and the within-ampoule homogeneity analysis. The homogeneity of the cod liver oil reference

material was hence considered to be suitable for use as CRM for organochlorine pesticides.

The stability of the organochlorine pesticides in the cod liver oil was studied at three temperatures (-20°C , $+20^{\circ}\text{C}$ and $+40^{\circ}\text{C}$) after 1, 3, 6 and 12 months, using the same method as applied in the homogeneity study. Due to an interference with toxaphene, the results of the measurement of γ -chlordane were found to be unrealistic. Therefore, the stability of this compound could not be demonstrated. As for o,p'-DDE the origin of the unreliability of the results is due to the instability of o,p'-DDT in the analytical system, both compounds were not considered for certification. For the remaining OCPs no instability could be demonstrated.

7.5.3. Certification

The final determination of the OCPs made by all the laboratories was based on capillary gas chromatography with either electron capture or mass spectrographic detection. Each laboratory used its own standard procedures regarding sample preparation, clean-up, method of injection, choice of carrier gas and chromatographic conditions. Each parameter was optimised by the participating laboratories prior to undertaking the determinations. Prior to the OCP determinations, participants were required to analyse an unknown solution containing the OCPs of interest for the certification. Data were submitted and the values of the OCPs in the solution were given to all participants with comments where necessary. If any result differed greater than $\pm 10\%$ from the target value, participants were asked to check their calibration solutions. Several sets were consequently withdrawn due to unreliable calibration.

Two calibration methods were recommended: multipoint calibration of at least four levels over the range of masses injected (each compound being quantified within the calibration range), and response factors used where the mass of the determinand was within ± 10 – 15% of the mass of the calibrant.

Participants were asked to check for the degradation of p,p'-DDT in the injector or on the column; they were required to submit a chromatogram of this compound on its own after optimisation of the GC and prior to the determination of the OCPs.

Prior to the determination, laboratories were also required to optimise analytical methodologies to ensure the quality of the data produced. In particular, the percentage recovery achieved for each of the certified OCPs had to be stabilised.

The different sample pretreatment (dissolution into various solvents), clean-up (using a variety of techniques to remove lipids and other interfering compounds), and GC-ECD or GC-MS conditions are described in detail in the certification report [16].

The determination of OCPs in a biological matrix requires the use of complex analytical procedures including numerous sources of systematic errors. Of the 15 compounds targeted for certification, thirteen were certified. For o,p'-DDE and o,p'-DDT only indicative values were given, owing to the possible degradation of DDT during the GC separation.

For the determination of all of the OCPs, a megabore column was not found to be suitable due to the poor chromatographic separation between determinands and between

determinands and interfering compounds. Data were accepted for certification only if the following criteria were met:

- (a) Recovery data should be >70%.
- (b) The standard deviation of the recovery data should be consistent with the standard deviation of the certification measurements.
- (c) There was no chromatographic interference present.
- (d) Data produced using two methods of detection (ECD and MS) within a single laboratory should be consistent.
- (e) Five laboratories could not fulfil the *a priori* requested conditions. Therefore, their data were not considered for certification.

The certified values of the 13 OCPs are given in the Table 7.10.

7.5.4. Participating laboratories

The project has been coordinated by the SOAFD Marine Laboratory in Aberdeen (United Kingdom) which also carried out the preparation of the material and the verification of its homogeneity and stability. The following laboratories participated in the certification: Agricultural Research Centre, Jokioinen (Finland); Institute of Marine Research, Bergen (Norway); Instituto Hidrográfico, Lisboa (Portugal); Laboratoire Central d'Hygiène Alimentaire, Paris (France); Milchwirtschaftliche Untersuchungs- und Versuchsanstalt, Kempten (Germany); Ministry of Agriculture, Fisheries and Food, Burnham-on-Crouch (United Kingdom); Nestec Ltd., Nestlé, Lausanne (Switzerland); RIVM, Bilthoven (The Netherlands); RIVO-DLO, Ijmuiden (The Netherlands); SOAFD Marine Laboratory, Aberdeen (United Kingdom); Statens

TABLE 7.10

CERTIFIED CONTENTS OF OCPs IN COD LIVER OIL CRM 598

Compound	Certified value ($\mu\text{g kg}^{-1}$)	Uncertainty ($\mu\text{g kg}^{-1}$)	No of accepted sets of results
HCB	55.7	2.0	11
α -HCH	42	3	13
β -HCH	16	3	9
γ -HCH	23	4	9
γ -chlordane	6.9	1.6	6
α -chlordane	24.4	1.8	12
Oxychlordane	11.0	1.8	7
Transnonachlor	39	4	12
Dieldrin	59	4	12
p,p'-DDE	0.61×10^3	0.04×10^3	9
o,p'-DDD	30	4	9
p,p'-DDD	0.40×10^3	0.03×10^3	10
p,p'-DDT	0.179×10^3	0.018×10^3	9

Lantbrukskemiska Laboratorium, Uppsala (Sweden); Teagasc, The National Food Centre, Dublin (Ireland); TNO-Voeding, Zeist (The Netherlands).

7.6. TRACE ELEMENTS IN MUSSEL TISSUE

7.6.1. Introduction

All Member States have regulations on the quality of the marine and aquatic environment, e.g. directives on the protection sea life in general. Marine monitoring involves the analysis of different compartments, e.g. water, sediment, biota. Water analysis is not sufficient to evaluate pollution trends since it gives only the concentration of a contaminant at the moment of sampling. Trends may hence be assessed by measuring the accumulation pattern in sediments or biological tissues and, for this purpose, fish, mussels and aquatic plants are frequently analysed, owing to their easy handling (sampling along the coast) and good indicator capabilities. The reference material described in this section has been prepared in the frame of a larger programme to establish a range of environmental CRMs for the quality control of marine monitoring.

7.6.2. Production of the material

Mussels (*Mytilus edulis*) were fished in the Dutch Waddensea. They were cooked under pressure (at ca. 120°C) for 30 s and the shells were removed. Then they were instantly deep-frozen (liquid nitrogen) and stored at a temperature below -28°C. Some 550 kg material was freeze-dried during 20 h and ground in a stainless-steel mill which could have caused some contamination with Fe. Upon freeze-drying, the material have lost 73.5% of its mass.

The material was further processed at the Joint Research Centre of Ispra where further grinding (ball mill in zirconia) was carried out to pass a sieve with apertures of 125 µm. The material was then mixed for two weeks under dry air (which was replaced twice a day) in a special polythene-lined mixing drum. After this period, bottling was performed in batches of 40. One bottle was selected randomly out of each batch and set aside for the homogeneity study.

The homogeneity was verified by determining the elements Ca, Cu, Fe, Mg, Mn, P, Zn, Cd and Pb at 50, 100 and 250 mg levels of intake. Determination were performed by ZETAAS (Cd, Pb) and ICP-AES (other elements) after pressurised digestion (10 h) with nitric acid at 160°C in quartz vessels. The homogeneity was demonstrated at a level of 100 mg and above [17].

The stability study was verified over 12 months at -20°C, +20°C and +40°C, monitoring the contents of matrix elements (C, H, P, N), minor elements (Na, Cl, Fe, Mn) and trace elements (Hg, Cd, Pb, Se). Measurement methods were INAA, catharometry after combustion in He/O₂ followed by conversion over Cu/CuO and pressurised digestion followed by ETAAS, CVAAS or ICP-AES. No instability was detected for any of the elements tested, even at prolonged storage at +40°C.

7.6.3. Certification

Eighteen laboratories from nine European countries participated in the certification (see section 7.3.9). The techniques of final determination are summarised in Table 7.11. Several types of sample pretreatment were used such as digestion with combination of acids in a pressurised or atmospheric mode, programmed dry ashing, combustion or irradiation with thermal neutrons [17].

The material contains high amounts of arsenobetaine which is more resistant to an oxidising acid attack than As-containing compounds in biota usually are. Preliminary investigations had shown that the digestion can only be complete (which is necessary for techniques such as HGAAS and ETAAS: possibly not fully mineralised arsenobetaine is not quickly reduced to AsH_3 and it might be swept out of the carbon oven of the ETAAS-system before the measurement stage) if oxidising acids including H_2SO_4 are used at temperatures of 160–200°C, depending on the kind of acids and amount. Dry ashing requires the use of ashing aids.

For Cd, laminar flow clean benches or clean rooms were recommended to avoid contamination. Acids used should be further purified by sub-boiling point distillation. If all precautions to reduce blanks are not followed too high results will be observed.

Determination of Se by HGAAS can be interfered by As if present at a higher level than usual in biota which may result in systematic errors if no corrections are applied (errors of approx. 10% were reported in the literature [17]).

The certified values are given in Table 7.12; indicative values are reported in the certification report [17].

The comparison of methods for some elements did not allow us to detect any bias due to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 7.13).

TABLE 7.11

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN MUSSEL TISSUE CRM 278

Elements	Techniques of final determination
As	EDXRF, ETAAS, HICP, HGAAS, RNAA, SPEC, ZETAAS
Cd	DPASV, ETAAS, IDMS, ZETAAS
Cr	ETAAS, ICP-AES, IDMS, INAA
Cu	DPASV, EDXRF, FAAS, ICP-AES, IDMS, RNAA, ZETAAS
Fe	EDXRF, FAAS, ICP-AES, IDMS, INAA
Hg	CVAAS, HICP, MIP-AES, RNAA
Mn	ETAAS, ICP-AES, INAA, ZETAAS
Pb	DPASV, EDXRF, ETAAS, FAAS, IDMS, ZETAAS
Se	EDXRF, ETAAS, FLUOR, HGAAS, HICP, IDMS, INAA, RNAA
Zn	DPASV, EDXRF, FAAS, ICP-AES, IDMS, INAA, PSA, SSAS, RNAA

TABLE 7.12
CERTIFIED CONTENTS OF TRACE ELEMENTS IN MUSSEL TISSUE CRM 278

Element	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
As	5.9	0.2
Cd	0.34	0.02
Cr	0.80	0.08
Cu	9.60	0.16
Fe	133	4
Hg	0.188	0.007
Mn	7.3	0.2
Pb	1.91	0.04
Se	1.66	0.04
Zn	76	2

7.6.4. Participating laboratories

The preparation of the material was carried out by the Delta Instituut voor Hydrobiologisch Onderzoek in Yerseke (The Netherlands) and the Joint Research Centre in Ispra (Italy). The homogeneity has been verified by the Gesellschaft für Strahlen- und Umweltforschung in Neuherberg (Germany), the Isotope Division of the Risø National Laboratory in Roskilde (Denmark) and the TNO Division of Society in Zeist (The Netherlands). Finally, the stability study has been performed by the Energieonderzoek Centrum Nederland in Petten (The Netherlands).

The following laboratories participated in the certification campaign: An Forais Taluntais, Wexford (Ireland); Aristotle University, Lab. of Analytical Chemistry (Greece); Centro di Radiochimica ed Analisi per Attivazione, Pavia (Italy); CNRS, Service Central d'Analyse, Vernaison (France); Delta Instituut voor Hydrobiologisch Onderzoek, Yerseke (The Netherlands); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Ecole Nationale Supérieure de Chimie, Strasbourg (France); Gesellschaft für Strahlen- und Umweltforschung, Neuherberg (Germany); Institut für Anorganische Chemie, Universität Regensburg (Germany); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Isotopcentralen, Copenhagen (Denmark); Joint Research Centre, Ispra (Italy); KEMA, Arnhem (The Netherlands); Kernforschungsanlage, Jülich (Germany); Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven (The Netherlands); Risø National Laboratory, Roskilde (Denmark); TNO Division of Technology for Society, Zeist (The Netherlands); Unità Locale Socio Sanitaria, Venezia (Italy).

7.7. ORGANOTIN COMPOUNDS IN MUSSEL TISSUE

7.7.1. Introduction

The toxic impact of tributyltin (TBT) on marine organisms is well known since the identification of harmful effects on oysters of Arcachon Bay (France) in the 1980's [18].

TABLE 7.13

COMPARISON OF METHODS FOR Cd, Cu, Fe, Mn, Pb AND Zn IN CRM 278

Element	Techniques	Number of labs	CV (%) (*)	CV (%) (**)
Cd	DPASV	6	11.5	3.2
	ETAAS	3	7.3	
	IDMS	2	4.3	
Cu	DPASV	5	3.5	1.7
	ICP-AES	5	4.1	
	AAS	3	2.3	
	IDMS	2	2.3	
	RNAA	3	4.2	
	AAS	3	2.1	
Fe	ICP-AES	4	4.5	3.4
	INAA	4	7.0	
	ICP-AES	5	7.9	
Mn	INAA	4	1.6	4.4
	DPASV	5	2.8	
Pb	ETAAS	3	6.1	1.1
	IDMS	3	0.25	
	ICP-AES	3	7.2	
Zn	AAS	3	2.2	2.3
	INAA	5	7.0	
	IDMS	2	6.1	

(*) CV between means of laboratories with the same technique

(**) CV between means of different techniques

This compound is released in the marine environment from the leaching of TBT-based antifouling paints used on boats and ships and its use has now been regulated in several countries. Other compounds are known to be very toxic to marine life, e.g. triphenyltin, and these compounds, along with their degradation products, are currently determined routinely by some laboratories to underpin controls of the levels of environmental contamination. These analyses are particularly requested in support of some EC directives [19].

A wide variety of analytical techniques has been developed within the last decade for the determination of organotins. Because these methods involve several analytical steps, such as extraction, derivatisation, separation and final detection, the risks of analytical errors are multiplied [20]. Some of these techniques are far from being validated. A programme for evaluating the performance of these methods has been organised by the BCR [21]. These interlaboratory studies concerned, in a first stage, intercomparisons to evaluate and improve the state of the art of TBT determinations in solutions and TBT-spiked sediment, and developed into two certification campaigns on butyltins in sediment (see Chapter 9). The need for a biological material certified for organotins was expressed by a group of European laboratories, the only material existing

(in limited stock) at this stage (in 1990) being the fish material NIES No. 11 from the National Institute for Environmental Studies, which was not considered to be sufficient to meet the demand. Consequently, the BCR decided to launch a project of which the aim was to certify butyl- and phenyltin in a candidate reference material (CRM 477). This section describes this certification.

7.7.2. Production of the material

In recent years, several monitoring campaigns were carried out in the La Spezia Gulf (Liguria, Italy) in order to study the environmental distribution and fate of organic micro-pollutants. The harbour of La Spezia is characterised by intense maritime traffic and dockyard activity (both civil and military) and by the presence of one of the most important Italian mussel farms. Analyses performed on mussels collected during these campaigns showed high contents of organotin compounds in mussel tissues as a consequence of maritime activities [22], which made samples from this area suitable as a candidate reference material for the certification of butyl- and phenyltin compounds.

1200 kg of mussels (*Mytilus edulis*) was purchased directly at the La Spezia mussel farm. After collection, the mussel samples were washed with fresh water to eliminate matrix salts, which could interfere in the preparation process or the analysis. The samples were immediately frozen by immersion in liquid nitrogen. Shelling could not be performed by cooking or using a vapour stream since this treatment could have caused degradation of organotin compounds. It was preferred to shell the frozen materials directly by using special mussel knives. The edible part was collected in thermally sealed polyethylene bags (ca. 4 kg per bag) and immediately stored at -25°C .

The frozen material (ca. 325 kg) was transported to the Biostarters Company (Parma) where it was ground, using a PTFE-coated mill, and spread on sterilised flat trays for the freeze-drying treatment. The process consisted of dividing the homogenate into batches, leaving the material at ca. -55°C for 6 h, then applying the vacuum for 48 h. Analyses were performed at the end of each freeze-drying process on samples collected from the top, the intermediate and the bottom levels of the flat trays, to evaluate the suitability of the process. The results showed that a moisture content of less than 4% was achieved. The freeze-dried material (final amount ca. 35 kg) was put into thermally sealed polyethylene bags, stored at -25°C and transported to the Joint Research Centre at Ispra.

The freeze-dried material was ground for 15 days in a zirconia ball mill, taking all precautions to avoid contamination. It was then sieved at 125 μm mesh with a titanium sieve in order to separate the fibrous part of the bulk material and mixed for 15 days under argon atmosphere in a special polyethylene-lined mixing drum. The argon atmosphere was renewed after 5 and 10 days. The material was bottled under argon atmosphere in brown Pyrex-glass bottles, remixing the sample for 30 min after 40 bottles had been filled. Bottles were set aside during the bottling procedure for the homogeneity and stability studies. One thousand bottles, each containing ca. 15 g, were obtained.

The homogeneity was verified by the determination of mono-, di- and tributyltin (MBT, DBT and TBT) and mono-, di- and triphenyltin (MPhT, DPhT and TPhT) on sub-samples of 500 mg. Organotin determinations were carried out as follows [22]: the

sample sub-sample (500 mg) was placed in a Pyrex vial (20 mL) and about 500 ng (as Sn) of internal standard (tripropyltin chloride in methanolic solution) was added. A mixture of methalonic tropolone and HCl was then added, and the vial, capped with a Teflon-lined screw cap, was placed in an ultrasonic bath for 15 min. The procedure was repeated twice, collecting the supernatant after centrifugation and transferring it to a 250 mL cylindrical separatory funnel. 25 mL of dichloromethane were added and the solution was shaken with 200 mL of a NaCl solution. This operation was repeated with another 25 mL of dichloromethane aliquot. The organic phases were collected through anhydrous sodium sulphate and concentrated down to few mL by a gentle stream of nitrogen and transferred to a 15 mL reaction vial. 2 mL of isooctane were added and the solution was brought near to dryness under moderate flow of nitrogen. One mL of an ethereal solution of pentylmagnesium bromide (2 mol L^{-1}) was then added, the vial was capped and the Grignard reaction was allowed to proceed by shaking in a thermostatic bath for 1 h, after which 2 mL of iso-octane were added. The excess reagent was destroyed by carefully adding about 1–2 mL of water (dropwise) and then 5–10 mL of sulphuric acid (1 mol L^{-1}). The organic layer was removed with a Pasteur pipette and put on the top of a 3 g silica-gel column for clean-up. The column was eluted with hexane:benzene (1:1) until 5 mL were collected. The solution was finally reduced to an exact volume (usually 1 mL) and 1 μL was injected into the GC-MS apparatus. The gas chromatograph was a HP 5890 (capillary column HP Ultra 2 25 m \times 0.2 mm \times 0.11 μm , helium as a carried gas). The detector was a HP 5970 MSD (EI quadrupole).

As mentioned above, quality control of organotins in biological materials is limited, owing to the lack of CRMs available (with the exception of NIES 11, certified for TBT and DBT). To ensure the best traceability of measurements, pentylated organotin calibrant solutions were prepared at the beginning of the study and stored at -20°C in the dark [21] to serve as independent control solutions, as the stability of fully derivatised organotins far exceeds that of the starting compounds, particularly for phenyltins. Freshly prepared stock and working calibrant solutions were checked for degradation products by GC-MS after pentylation and analysed with respect to the stored pentylated calibrant solutions. In order to control the method performance, organotin working calibrant solutions were regularly run through the whole analytical procedure. Single analytical steps were carefully checked for performance problems or relevant changes in the materials used (e.g. clean-up for new batches of silica gel). Recovery tests were performed using freeze-dried mussel samples analysed before and after spiking with ca. 160 ng (as Sn) of each butyl- and phenyltin compounds [22]. Organotins were added as solutions in methanol to the samples previously wetted with distilled water; after the addition, the mussel samples were shaken for at least 30 min and allowed to equilibrate overnight. The recoveries were calculated with respect to the sum of the contents of the incurred compounds and the spikes. Recoveries ranged from $(80 \pm 15)\%$ to $(90 \pm 10)\%$ (Table 7.14).

The homogeneity of the three butyltin species (MBT, DBT and TBT) was found to be sufficient at a level of 500 mg and above. In the case of phenyltin species, however, the homogeneity of the compounds was found to be acceptable at the time of the sample

TABLE 7.14

RECOVERIES OF ORGANOTIN COMPOUNDS FROM 500 MG OF NON-SPIKED AND SPIKED MUSSELS. RESULTS ARE THE AVERAGE OF FIVE DIFFERENT EXPERIMENTS [8]

Compound	Non-spiked (ng Sn)	Spiked amount (ng Sn)	Found (ng Sn)	Recovery (%)
TBT	185 ± 24	160	314 ± 27	91 ± 9
DBT	61 ± 10	165	201 ± 23	89 ± 11
MBT	80 ± 13	150	195 ± 29	85 ± 15
TPhT	nd	150	138 ± 13	92 ± 9
DPhT	nd	157	133 ± 18	85 ± 14
MPhT	nd	147	120 ± 20	82 ± 17

nd: not detected

preparation but instability problems (see section 7.7.4) created in-homogeneities at a later stage (likely due to different degradation rate from bottle to bottle).

The stability was studied at -20°C , $+4^{\circ}\text{C}$, $+20^{\circ}\text{C}$ and $+40^{\circ}\text{C}$ over a period of 12 months by the determination of organotins at regular intervals. Tests were repeated after 24, 36 and 44 months storage at -20°C . This study showed that instability risks existed for long term storage at $+20^{\circ}\text{C}$ and that butyltins were not stable at $+40^{\circ}\text{C}$; however, the three compounds were shown to be stable at -20°C , which made the reference material suitable for use as a CRM, providing that particular care be taken in terms of storage. Stability checks carried out on phenyltins clearly showed that the three compounds (mono-, di- and triphenyltin) were not stable at any of the temperatures tested; consequently, it was decided not to give any indicative values for these compounds [23].

7.7.3. Certification

Fourteen laboratories from seven European countries participated in the certification (see section 7.4.7). The techniques used were based on hyphenation of different extraction, chromatography and detection methods. The separation and detection methods are given as abbreviations in Table 7.15. The sample pretreatment methods were based on digestion by e.g. tetramethyl ammonium hydroxide (with or without leaching), protease/lipase (enzymatic digestion), HCl/diethylether/NaCl; extraction with e.g. methanol, tropolone or acetic acid; and/or supercritical fluid extraction with CO_2 . Derivatisation was applied in some cases either using NaBH_4 or NaBEt_4 , or by Grignard reactions. A full description of the techniques used is given in the certification report [23]. It should be noted that a set of pure calibrants (butyltin chloride compounds, 'secondary' calibrants such as pentylated or ethylated butyl- and phenyl-tin compounds) was prepared for the purpose of this certification; these compounds were used as primary

TABLE 7.15

SUMMARY OF TECHNIQUES USED IN THE CERTIFICATION OF BUTYLTINS IN MUSSEL TISSUE CRM 477

Compounds	Techniques of separation and final determination
MBT, DBT and TBT	HG/GC/QFAAS, HG/CGC/FPD, HPLC/FLUO, HPLC/ICP-AES, HPLC/ICPMS, HPLC/ID-ICPMS, Et/GC/QFAAS, Et/CGC/MIP-AES, Et/CGC/FPD, Et/CGC/MS, Pe/CGC/FPD, Pe/CGC/MS

calibrants and to verify the yield of the derivatisation reactions. This procedure enhanced considerably the traceability of the results and their overall comparability.

Differences in standard deviations between two HPLC-ICPMS sets for tributyltin were due to the fact that the lower SD was obtained by an isotope-dilution methodology.

In the case of dibutyltin, relatively high results with a large CV were obtained by HPLC-ICP-AES and doubts were raised about the specificity of the liquid/liquid extraction procedure used which could have led to an over-estimate of the values due to uncertainties in recovery; the set was therefore withdrawn.

Low extraction recoveries were observed in some cases for monobutyltin, which justified the rejection of sets of results.

Evaluations of triphenyltin results brought the following comments: the effect of use of methanol and drying (blowing down) on derivatisation (Grignard reaction) could lead to degradation of compounds, leading to higher analytical uncertainties (this effect could be photo-induced); it was recommended to perform the extraction and derivatisation in a single step process.

The spread of results of mono- and diphenyl-tin was much too high to draw any conclusions, except that the state of the art is not sufficient at this stage to enable certification (both owing to instability problems and lack of agreement among techniques/laboratories).

The certified values of butyl-tin compounds are given in Table 7.16. The three compounds are certified as mass fractions mg kg^{-1} of $\text{Sn}(\text{C}_4\text{H}_9)^{3+}$ (MBT), $\text{Sn}(\text{C}_4\text{H}_9)_2^{2+}$ (DBT) and $\text{Sn}(\text{C}_4\text{H}_9)_3^+$ (TBT).

TABLE 7.16

CERTIFIED CONTENTS OF MBT, DBT AND TBT IN MUSSEL TISSUE CRM 477

Compound	Certified value (mg kg^{-1}) as cations	Uncertainty (mg kg^{-1}) as cations
MBT	1.50	0.27
DBT	1.54	0.12
TBT	2.20	0.19

7.7.4. Participating laboratories

The material was prepared by the Department of Environmental Chemistry of the ENEA in Roma (Italy) and the Joint Research Centre in Ispra (Italy). The homogeneity and stability studies were carried out by ENEA.

The following laboratories participated in the certification campaign: CID-CSIC, Department of Environmental Chemistry, Barcelona (Spain); ENEA, Divisione di Chimica Ambientale, Roma (Italy); GALAB, Geesthacht (Germany); Ministry for Agriculture, Fisheries and Food, Burnham-on-Crouch (United Kingdom); Rijks-waterstaat, RIKZ, Haren (The Netherlands); Universitaire Instelling Antwerpen, MITAC, Wilrijk (Belgium); Universidad de Barcelona, Depto. de Química Analítica (Spain); Université de Bordeaux I, Lab. Photophysique et Photochimie Moléculaire, Talence (France); Università di Genova, Dipto. di Chimica (Italy); Université de Pau, Lab. de Chimie Analytique (France); University of Plymouth, Dept. of Environmental Sciences (United Kingdom); Universidad de Huelva, Depto. de Química (Spain); Universidad de Santiago de Compostella, Depto. de Química Analítica (Spain); Vrije Universiteit Amsterdam, IVM (The Netherlands).

7.8. TRACE ELEMENTS IN PLANKTON

7.8.1. Introduction

Analyses of phyto- and zooplankton are important to investigate the transfer and uptake of toxic elements along the trophic chain, to define the role of these organisms in the biogeochemical pathways. Furthermore, plankton may be used as an indicator for water pollution and to compare the levels of contamination of different aquatic environments. The determination of trace elements is still troublesome as shown in an intercomparison between 18 laboratories on trace elements in plankton [24], where the presence of systematic errors was demonstrated. The quality of analysis of plankton material cannot be controlled using other biological CRMs owing to its composite nature (mixture of sedimentary and biogenic particles). To control the quality of trace element determinations in plankton, the BCR has therefore developed a plankton material which has been certified for trace elements of most interest, e.g. As, Cd, Cr, Cu, Fe, Hg, Ni, Mn, Pb, Se, V and Zn.

7.8.2. Production of the material

The plankton was collected from several ponds situated in the vicinity of the river Po. The ponds were fed by the Po water downstream to a power plant. The relatively high water temperature accelerated plankton growth, allowing the collection of the total amount necessary for the reference material within a relatively short period. The collection was done with a manually-operated net with 125 μm apertures. The wet material collected was packed in double-layer polythene bags and kept at -20°C until freeze-drying.

When sufficient wet materials was collected, it was freeze-dried, ground in a zirconia ball mill and sieved to pass 125 μm apertures. The resulting powder was collected in a mixing drum and homogenised by continuous rotation under dry Ar over two weeks. Cleaned brown glass bottles were filled with approximately 5 g of material and closed with screw caps and plastic inserts. A PTFE-ball was added to facilitate later re-homogenisation in case segregation would occur.

A microscopical examination of the material was carried out in order to establish a classification of particles (types and sizes), showing that the large majority of organisms (>98%) were cladocerans mainly of the species *Daphnia magna* with the occasional presence of a few copepods and rotifers [25].

A first series of tests was performed on major elements (C, N and H) at levels of intakes of 0.5 and 2 mg analysed with a C-H-N elemental analyser; no inhomogeneity was detected. A more extensive homogeneity study was carried out by the determination of As, Cd, Co, Cr, Cu, Fe, Hg, Mg, Pb, Se and Zn on intakes of 50, 100 and 250 mg. Analyses were performed by CVAAS (Hg), HGAAS (As and Se), ETAAS (Cd and Pb) and ICP-AES (Co, Cr, Cu, Fe, Mg and Zn) after pressurized digestion with nitric acid in a quartz vessel at 170°C for ca. 8 h. No systematic differences were observed between the CVs of the methods and the within- and between-bottle CVs and the material was hence considered to be homogeneous at a level of 50 mg and above. Additional tests were performed to assess possible segregation effects upon transport. Plankton material was loaded in a plastic tube, which was mounted vertically and gently vibrated at a 50 Hz frequency for 100 h; the tube was then cut in three sections, i.e. bottom, middle and top. The study of possible segregation was performed on the basis of the variations of the particle distribution (by microscopical examination). The results (Table 7.17) indicate that the particle sizes are decreasing towards the bottom of the tube after vibration, which clearly demonstrates that segregation may occur upon transport [24,25]. The sample homogeneity with regard to particle size distribution was verified again after manually shaking the bottle that showed segregation. No significant differences were observed between bottom, middle and top. This study highlighted the need to carefully shake the samples prior to analysis to avoid possible inhomogeneity due to segregation.

The stability of the material was tested at -20°C, +20°C and +40°C over a period of 12 months by determining a series of elements (As, Br, Co, Cr, Fe, Na, Mn, Se and Zn) at regular intervals over the storage period. Analyses were carried out by INAA.

TABLE 7.17

SIMULATION OF TRANSPORT EFFECTS (SEGREGATION TESTS) FOR PLANKTON CRM 414

	Segregation tests Mean (μm)	Homogeneity tests Mean \pm SD (μm)
Top	3.89	
Middle	2.96	2.894 \pm 0.003
Bottom	2.63	

No instability could be demonstrated and the material was hence kept stored at ambient temperature.

7.8.3. Certification

Fifteen laboratories from eight European countries participated in the certification (see section 7.6.4). Table 7.18 summarises the different techniques of final determination used; pretreatment techniques were based on digestion with combination of acids in pressurised or atmospheric mode, programmed dry ashing, combustion and irradiation with thermal neutrons. A detailed description of the methods is given in the certification report [24].

Due to the presence of clay particles, risks of incomplete mineralisation were likely; therefore, the participants were requested to indicate whether they had used HF for the digestion of the material or could prove that the total element content had been measured (e.g. by checking the residue).

The deuterium background correction was not recommended for the AAS determination of Cr in this materials since the intensity of the lamp is already very low at the wavelength used (276 nm).

The certified values are listed in Table 7.19. Indicative values are included in the certification report and published elsewhere [24,25].

The effects of HF-treatment have been investigated for Cd, Cr, Cu, Mn, Pb and Zn; no major difference (within the uncertainty of the method) between the digestion with or without HF was observed for Mn and Zn. After HF treatment, however, contents of Cu and Pb were significantly higher (respectively 6.4 and 9.6%) whereas Cd and Cr contents considerably increased by respectively 27% and 26%. In addition, an independent study to investigate the suitability of microwave digestion for this material, using an

TABLE 7.18

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN PLANKTON CRM 414

Elements	Techniques of final determination
As	HICP, HGAAS, ICPMS, RNAA, SPEC, ZETAAS
Cd	DPASV, ICPMS, IDMS, MS, ZETAAS
Cr	DPASV, ETAAS, ICP-AES, INAA, RNAA, ZETAAS
Cu	DPASV, FAAS, ICP-AES, ICPMS, MS, RNAA, ZETAAS
Hg	CVAAS, HICP, RNAA
Mn	ETAAS, FAAS, INAA, ICPMS
Ni	CSV, DPASV, ETAAS, ICP-AES, INAA, ZETAAS
Pb	DPASV, ETAAS, ICPMS, IDMS, ZETAAS
Se	DPCSV, HGAAS, HICP, INAA, MS, ZETAAS
V	DPASV, ICP-AES, INAA, ZETAAS
Zn	DPASV, FAAS, ICP-AES, ICPMS, IDMS, INAA, MS

TABLE 7.19

CERTIFIED CONTENTS OF TRACE ELEMENTS IN PLANKTON CRM 414

Element	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
As	6.82	0.28
Cd	0.383	0.014
Cr	23.8	1.2
Cu	29.5	1.3
Hg	0.276	0.018
Mn	299	12
Ni	18.8	0.8
Pb	3.97	0.19
Se	1.75	0.10
V	8.10	0.18
Zn	112	3

open system with two different programmes [3] which showed the suitability of this technique for the elements tested (Cd, Cu, Mn, Pb and Zn).

The comparison of methods for some elements did not allow us to detect any bias due to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 7.20).

7.8.4. Participating laboratories

The material was collected by the CNR Institute for Limnology in Pallanza (Italy) and prepared by the EC Joint Research Centre of Ispra (Italy). The homogeneity and stability studies were carried out at the GSF-Forschungszentrum für Umwelt und Gesundheit in Oberschleißheim (Germany) and at the Energieonderzoek Centrum

TABLE 7.20

COMPARISON OF METHODS FOR Cu AND Pb IN CRM 414

Element	Techn. of final determination	CV % between means of lab. with the same technique	Nr of sets or results	CV % between means of diff. techniques
Cu	FAAS	4.0	3	5.8
	RNAA	2.8	3	
	DPASV	10.7	3	
Pb	ZETAAS	4.7	3	3.4
	DPASV	8.3	3	

Nederland in Petten (The Netherlands). The microscopical examination of the material was performed at the Universitaire Instelling Antwerpen in Wilrijk (Belgium).

The following laboratories participated in the certification: Aristotle University, Lab. Analytical Chemistry, Thessaloniki (Greece); Danish Isotope Centre, Copenhagen (Denmark); Energieonderzoek centrum Nederland, Petten (The Netherlands); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Istituto Superiore di Sanità, Roma (Italy); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Kernforschungsanlage, Jülich (Germany); Labor für Spurenanalytik, Bonn (Germany); National Food Administration, Uppsala (Sweden); NCR 'Demokritos', Agia Parsakai Attikis (Greece); Presidio Multizonale di Prevenzione, Sezione Chimica, Venezia (Italy); Risø National Laboratory, Roskilde (Denmark); Universidad Complutense, Facultad de Química, Madrid (Spain); Università degli Studi di Pavia, Centro di Radiochimica Generale, Pavia (Italy); Universitaire Instelling Antwerpen, Wilrijk (Belgium).

7.9. PESTICIDES IN ANIMAL FAT

7.9.1. Introduction

Organochlorine pesticides (OCPs) belong to the class of chlorinated hydrocarbons that comprises compounds such as DDT and its degradation products, the cyclodiene compounds, HCB and the HCH isomers. Most of these are persistent and lipophilic compounds. They are not readily degraded in the environment, nor are they completely metabolised or excreted by organisms; instead, they bioconcentrate in the food chain and are stored in fat. They are hardly excreted under physiological circumstances, and hence are of substantial interest when considering food of animal origin.

Since the discovery of DDT as an insecticide in 1940, many OCPs have been developed and widely used in agriculture and animal husbandry. Although the use of most OCPs is restricted or banned in the EC from the early 1970s, residues are still being found in animal products, mainly due to the use of imported feed.

Certification of OCPs in pork fat was considered to an important step in support of quality control as an aid to ensure accuracy for the control of maximum residue limits in fat and food with a high fat content, the identification of pollution sources (e.g. certain feed ingredients) and studies of environmental trends. Ten OCPs most frequently found in fat (or food with high fat content) were selected and certified in pork fat packed in sealed ampoules (CRM 430) [26].

7.9.2. Production of the material

Pigs were fed for 14 weeks with feed enriched with organochlorine pesticides (OCPs). The pesticides used were pure calibrants of HCB, α -HCB, β -HCB, γ -HCB, heptachlor, dieldrin, endrin, and p,p' -DDT. Quantities of 100 mg to 1.8 g of these compounds were weighed and dissolved in hexane/maize oil. For β -HCB, acetone/maize oil was used. The desired amounts were calculated using maximum levels for fat applicable in EC

countries [25] and the accumulation factors described in the literature [27]. The solutions were mixed with the feed.

After slaughtering the pigs the fat was ground, melted down and dried under a constant stream of dry N_2 . The filtered material was stabilised by adding 0.02% (mass fraction) BHT antioxidant. The fat was homogenised in a stainless steel drum by stirring under argon at 50°C for 30 minutes. Then 50 ampoules of brown glass were filled with about 3 g fat and sealed. After re-homogenisation under argon for 30 minutes at 50°C a further batch of 50 ampoules was filled. The cycle was repeated until in total, about 1250 ampoules were produced.

Heptachlor was totally metabolised into b-heptachlor epoxide and p,p'-DDT partially metabolised into p,p'-DDE and p,p'-TDE. So, instead of eight OCPs the pork fat contained ten compounds: hexachlorobenzene, a-HCH, b-HCH, γ -HCH, b-heptachlor epoxide, p,p'-DDE, dieldrin, endrin, p,p'-TDE and p,p'-DDT.

The homogeneity was then verified for these compounds (between-ampoule homogeneity) after melting the fat at 50°C, sub-sampling 1 g fat and dissolving into 10.0 mL hexane. An aliquot of 1 mL of this extract was transferred to a basic alumina column (10 g, deactivated with 10 mg water per g alumina). The column was eluted with hexane. Two fractions of 10 mL and 100 mL were collected. After adding CB-207 (2,2',3,3',4,4',5,6,6'-nonachlorobiphenyl) as an internal standard for the gas chromatographic separation, both fractions were adjusted to a volume of 10.0 mL. The OCPs were determined by GC (splitless injection) on two fused silica capillary columns [26,28]. The CV of the analytical method and the between-ampoule homogeneity did not differ consistently; it was hence concluded that the pork fat is sufficiently homogeneous to serve as a CRM.

The stability of OCPs in the pork fat was tested at -20°C, +20°C and +37°C over a period of 12 months, using the same method as in the homogeneity study. Calibrant solutions were injected to check the performance of the GC system through all series of analyses. In addition, cleaned extracts of pork fat were prepared, set aside at the beginning of the study and kept at -20°C; they were used to control the long term reproducibility of the GC separation and the final detection. The OCP contents did not show any significant changes during the 12-month period, with the exception of some variations likely due to analytical artefacts [26,28].

7.9.3. Certification

The method of analysis for the final determination of these OCPs was based on gas chromatography with electron capture detection. Most of the participating laboratories used capillary GC. Mass spectrometry was used as a complementary technique but was not used to obtain quantitative data for the certification exercise. Each laboratory used its own procedures for sample preparation, extraction, clean-up, method of injection, choice of carrier gas, columns and other chromatographic conditions. Critical parameters were optimised by the analyst prior to undertaking these determinations [26].

Extractions were performed with different solvents after melting the fat at 40°C, e.g. petroleum ether, hexane or iso-octane. Various clean-up techniques were used to remove the fat and other compounds that could interfere with GC (either by masking the GC

response or by degrading the performance of the instrument), e.g. gel permeation chromatography, alumina column, silica gel, or Florisil. Beside the majority of capillary GC, some participants used widebore or packed columns; each OCP was identified and confirmed using at least two columns with different stationary phases. Recovery experiments were carried out, using blank pork fat; all results were corrected for recovery.

Each laboratory received calibration solutions prepared gravimetrically with OCPs of certified purity [26] and was requested to check its own calibrant with this solution or to use it for calibration. The choice of internal standards for extraction and GC was left to each participant; these were e.g. aldrin, α -HCH, isodrin, CB 88, CB 118, DCBE-C7 and CB 207 [26].

There was evidence from one laboratory that the extraction and clean-up procedure led to low recoveries for almost all OCPs; the data from this laboratory were therefore excluded. Laboratories whose results were accepted for certification all had recoveries between 70 and 120% [26].

The certification of endrin was based on the data from five laboratories only. The data from the other laboratories were rejected following a detailed inspection of the chromatograms, which showed that these laboratories were unable to achieve the chromatographic resolution or which showed a too high limit of detection for this compound (less than 20 times the standard deviation of the signal of the background signal under the peak).

A lack of agreement hampered certification of p,p' -TDE; indicative values are given in the certification report [26]. The certified values are listed in Table 7.21.

7.9.4. Participating laboratories

The material was prepared by TNO-CIVO Biotechnology and Chemistry Institute in Zeist (The Netherlands) which also performed homogeneity and stability studies, and the TNO-CIVO Toxicology and Nutrition Institute in Wageningen (The Netherlands).

TABLE 7.21

CERTIFIED CONTENTS OF ORGANOCHLORINE PESTICIDES IN PORK FAT CRM 430

Compound	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
HCb	0.392	0.026
α -HCH	0.140	0.012
β -HCH	0.259	0.021
γ -HCH	0.50	0.03
β -Hepo	0.109	0.009
Dieldrin	0.124	0.011
Endrin	0.020	0.004
p,p' -DDT	3.40	0.18
p,p' -DDE	0.82	0.06

The following laboratories participated in the certification: Chemisches Landesuntersuchungsamt, Münster (Germany); Food Inspection Service, Utrecht (The Netherlands); Laboratoire Central d'Hygiène Alimentaire, Paris (France); Nestec Ltd, Vevey (Switzerland); Shell Research Ltd, Sittingbourne (United Kingdom); Staatliches Veterinäruntersuchungsamt, Hannover (Germany); State Institute for Quality Control of Agricultural Products (RIKILT), Wageningen (The Netherlands); National Food Center, Dublin (Ireland); TNO-CIVO Biotechnology and Chemistry Institute, Zeist (The Netherlands).

7.10. MAJOR AND TRACE ELEMENTS IN MILK

7.10.1. Introduction

Milk and milk products form an important part of the human diet. They are analysed frequently for various components, e.g. for nutritional studies, and to comply with legislation controlling potential exposure to toxic elements. This section describes the preparation of a milk reference material (CRM 063R) which has been certified for a range of major and trace elements in replacement of a previous material (CRM 063) [29,30].

7.10.2. Production of the material

About 1200 kg of skimmed raw milk was collected and pasteurised by heating at 85°C for 60 seconds and then concentrated to a total solid content of 45% (mass fraction) in a four stage falling film evaporator. The concentrate was then spray-dried in a stainless steel drying tower using a nozzle sprayer; it was warmed to 50°C just before entering the nozzle. Approximately 100 kg of finely divided free flowing powder was collected in 4 bags each containing ca. 25 kg. The bags consisted of 4 separate layers: the inner bag was made of polythene, the three other layers were from paper coated with polyethylene. The bags were sealed immediately after filling and transferred to IRMM in Geel (Belgium). Precautions to prevent contamination or biological degradation of the sample were taken at all stages of preparation.

During the collection of the material, three samples were taken at the beginning, middle and end of the procedure for moisture determination by Karl Fischer titration. Furthermore, samples were taken from each bag before sealing and analysed for their microbe content and insolubility index at 24°C. The results indicated that no microbial contamination occurred during the preparation of the material and that the moisture content achieved (ranging from 2.4 to 3.3% mass fraction) was suitable to avoid microbiological growth [30,31].

The material was homogenised in a 60 L polyethylene drum for 2 h at 13 rpm and transferred to a dry atmosphere storage box. The material was then bottled in pre-cleaned brown glass bottles of 100 mL with polyethylene inserts, each containing ca. 50 g of milk powder.

The homogeneity was verified by the determination of Kjeldahl-N, P, Cl, K, Ca, Fe,

Cu and Zn at sample intakes of 100–500 mg and 250–1250 mg (depending on the analyte). Kjeldahl-N was determined in accordance with the method given by the International Dairy Federation (20:1986) [30]; in this method, the digestion is carried out in specially designed tubes with a mixture of H_2SO_4 and H_2O_2 with $\text{K}_2\text{SO}_4/\text{CuSO}_4$ as a catalyst. After elution, the NH_4 in the digest reacts with an alkaline mixture of sodium salicylate and sodium dichloroisocyanurate. The green coloured reaction product was measured spectrometrically at 660 nm. Determinations of P and Fe were performed after digestion with concentrated $\text{H}_2\text{SO}_4/\text{HNO}_3$ and addition of H_2O_2 . In the case of P, ammonium molybdate was added and P was determined by measurement of the absorbance of the molybdenum blue complex at 820 nm. For Fe, Fe(III) was reduced with hydroxyl ammonium chloride to Fe(II) which was complexed with batho-phenanthroline and the coloured complex absorbance was measured at 533 nm. Chloride was determined by titration with thiocyanate using Fe(III) as an indicator, after addition of AgNO_3 and concentrated HNO_3 , followed by KMnO_4 addition. Ca and K were determined by FAAS and FAES, respectively, after dispersion of the milk sample in water and addition of SrCl_2 (for Ca) and CsCl (for K). Cu and Zn were determined by ZETAAS after addition of $\text{Pb}(\text{NO}_3)_2$ and $\text{Mg}(\text{NO}_3)_2$ matrix modifiers in the case of Cu, and addition of SrCl_2 in the case of Zn. The results of the study showed that the material is homogeneous at least at a level of 100 mg and above for Kjeldahl-N, Ca, K and P; 200 mg and above for Zn; 400 mg and above for Fe and 500 mg and above for Cl (minimum sample intake for the method used). In the case of Cu, an inhomogeneity was suspected at a level of 200 mg but the material was found to be homogeneous at the 500 mg level for this element.

A micro-homogeneity study was carried out by solid sampling ZETAAS for Pb and Zn which showed that the material may be considered as homogeneous for these two elements at levels of 10 mg (Pb) and 87 mg (Zn) and above [31].

The first milk material produced by BCR (CRM 063) was shown to be stable for over ten years. Nevertheless, a study was carried out to confirm the stability of the present material (CRM 063R). Tests were performed at -25°C , $+4^\circ\text{C}$ and $+25^\circ\text{C}$ over a period of 12 months and Kjeldahl-N, P, Cl, K, Ca, Fe, Cu and Zn were determined. Experience has shown that heating at temperatures above 28°C for a longer period of time is disadvantageous because of the Maillard-reaction that occurs (reaction between proteins and sugars) and causes the material to turn brown and form lumps. Although the content of the elements of concern does not change, their speciation may be modified and the material has to be ground which certainly may increase the risks of contamination by trace elements. The material was shown to be stable at the three temperatures tested for all the elements of concern [30]. In addition, a short-term stability study at $+45^\circ\text{C}$ for a period of 14 days for the assessment of worst-case conditions upon transport showed that the element contents were not affected significantly.

7.10.3. Certification

Seventeen laboratories from eleven European countries participated in the certification (see section 7.9.7). Table 7.22 summarises the different techniques of final determination used; pretreatment techniques were based on digestion with combination of acids in

TABLE 7.22

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF MAJOR AND TRACE ELEMENTS IN MILK POWDER CRM 063R

Elements	Techniques of final determination
Ca	DCP-AES, FAAS, GRAV, ICP-AES, IDMS, INAA
Cl	IC, INAA, TVOL, TPOT
Mg	DCP-AES, FAAS, ICP-AES, IDMS, INAA, ZETAAS
Total-N	CSCAT, GCCAT
P	GRAV, RNAA, SPEC, TAC
K	DCP-AES, FAAS, FAES, FPD, ICP-AES, INAA
Na	DCP-AES, FAAS, FAES, ICP-AES, INAA
Cu	DCP-AES, DPASV, ETAAS, ICP-AES, ICPMS, RNAA, SPEC, ZETAAS
Fe	DCP-AES, ETAAS, ICP-AES, IDMS, INAA, SPEC, ZETAAS
I	ICPMS, RNAA
Pb	DPASV, ICPMS, IDMS, ZETAAS
Zn	DCP-AES, DPASV, FAAS, ICP-AES, IDMS, INAA

pressurised or atmospheric mode, programmed dry ashing, combustion and irradiation with thermal neutrons. A detailed description of the methods is given in the certification report [30].

Very small standard deviations were observed for chloride results obtained by potentiometric titration, which is inherent to the technique.

In the case of total nitrogen, two laboratories used a gas chromatographic separation of CO₂, H₂O and N₂ after combustion (GCCAT); two other laboratories trapped the combustion gases chemically and measured the remaining N₂ (CSCAT). Since the major sources of error in the procedures are the conversion and the separation, CSCAT and GCCAT were considered to be independent methods, although in both cases the final signal was obtained by catharometry.

The high spread of results obtained for Kjeldahl-N with the IDF-20A method [1] could not allow this component to be certified. An indicative value is given in the certification report [30].

Results obtained with plasma techniques for phosphorus could not underly certification for it could not be concluded from the description of the methods that the calibration had taken place under exactly the same conditions as the determinations (e.g. temperature, matrix components or that the calibrant solutions were not matrix-matched); these results were given for information only in the certification report [30].

A large variance for Na by INAA could be explained by the application of a low neutron flux and short irradiation and measuring times; the uncertainty was related to the statistics of counting low numbers. A large standard deviation was also observed for Fe, also dictated by counting statistics.

A value obtained by SSZETAAS for Pb was given as indicative but was not used for the calculation of the certified value since this method was not considered to be sufficiently traceable for certification at this stage.

The certified values are given in Table 7.23. Indicative values are given in the certification report [30].

The comparison of methods for some elements showed that no particular bias could be attributed to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 7.24)

TABLE 7.23A

CERTIFIED CONTENTS OF MAJOR ELEMENTS IN MILK POWDER CRM 063R

Element	Certified value (g kg ⁻¹)	Uncertainty (g kg ⁻¹)
Ca	13.49	0.10
Cl	9.94	0.30
K	17.68	0.19
Mg	1.263	0.024
Total-N	62.3	0.8
Na	4.37	0.03
P	11.10	0.13

TABLE 7.23B

CERTIFIED CONTENTS OF TRACE ELEMENTS IN MILK POWDER CRM 063R

Element	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
Cu	0.602	0.019
Fe	2.32	0.23
I	0.81	0.05
Pb	0.0185	0.0027
Zn	49.0	0.6

7.10.4. Participating laboratories

The material has been prepared by the Netherlands Institute for Dairy Research (NIZO) in Ede (The Netherlands), which also carried out homogeneity and stability studies, and the Institute for Reference Materials and Measurements in Geel (Belgium). The following laboratories participated in the certification campaign: Agriculture and Food Laboratory, Santander (Spain); Aristotelian University, Lab. of Analytical Chemistry, Thessaloniki (Greece); Laboratoire Central d'Hygiène Alimentaire, Paris (France); CNRS, Service Central d'Analyse, Vernaison (France); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Istituto Superiore di Sanità, Roma (Italy); LNETI, Dept. of Technology and Food Industry, Lisbon (Portugal); Milk Marketing Board, Thames-Ditton (United Kingdom); Milk Research Institute (MUVA), Kempten (Germany); MT-TNO, Zeist (The Netherlands); Portsmouth Polytechnic

TABLE 7.24

COMPARISON OF METHODS FOR Cl, Mg, Cu AND Zn IN CRM 063R

Element	Techniques	Number of labs	CV (%) (*)	CV (%) (**)
Cl	INAA	3	1.8	3.9
	TVOL	3	2.0	
Mg	FAAS	6	2.7	1.5
	INAA	3	2.2	
Cu	RNAA	3	5.2	6.7
	ZETAAS	3	5.8	
Zn	FAAS	5	2.5	0.1
	INAA	3	2.5	

(*) CV between means of laboratories with the same technique

(**) CV between means of different techniques

(United Kingdom); Universiteit Gent, I.N.W. (Belgium); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Risø National Laboratory, Roskilde (Denmark); Swedish Dairy Association, Lund (Sweden); Università di Pavia, Centro di Radiochimica (Italy); University of Plymouth, Department of Environmental Chemistry (United Kingdom).

7.11. CBs IN MILK

7.11.1. Introduction

Chlorobiphenyls (CBs) are used widely by industry, including transformers and capacitors in the electrical industry and inks, paints and paper in the printing and allied industries. Unwanted polychlorinated biphenyls (PCB) formulations and waste materials containing PCBs are normally incinerated. However, PCBs and waste products containing these materials are inadvertently or illegally disposed of at dump sites and landfill sites. Unsealed PCB sources are leached into the terrestrial and aquatic environment and incomplete combustion vaporises these compounds into the atmosphere; therefore, they enter the wider global cycle of trace organic compounds and ubiquitous environmental contaminants that occur in bovine milk, milk powders and products as a result of intake via animal feed.

Of the possible 209 CB congeners, a range of compounds are generally determined in various environmental compartments, owing to their presence in industrial mixtures, toxicity, legislation, persistence in the environment, e.g. the CBs 28, 52, 101, 118, 138, 153 and 180. With respect to the monitoring of milk or milk products, the relative concentrations of the CBs found in industrial formulations are modified by mammalian metabolism of the lower chlorinated (less than 5 Cl) congeners. In the view of producing a milk powder reference material certified for its CB content, additional congeners were therefore selected and a material (CRM 450) was certified for its content of CBs 52, 118, 153, 156, 170 and 180 [32].

7.11.2. Production of the material

The milk powder was obtained from a mixture of a full cream milk (125 kg) containing a moderate level of PCBs, a full cream milk (190 kg) with very low levels of PCBs and a skimmed milk (140 kg), also with very low levels of PCBs. All the milk was obtained from mid-southern Germany. The mixed milk was heat-treated at 85°C for 30–60 seconds in a plate heat exchanger and then condensed at 45–62°C to 38% dry mass in a double effect evaporator. The condensed milk was spray dried at 170–195°C in a pilot dryer with concurrent flow. Ten batches of 3–4 kg powder were sealed into polyethylene (PE) bags.

The milk powder from these 10 batches was homogenised in a mixing drum. Brown glass ampoules, cleaned with n-hexane, were filled with 20–23 g of milk powder, flushed with argon and sealed. In total 21 batches with 65 ampoules in each were produced. The ampoules were stored in the dark at $(5 \pm 2)^\circ\text{C}$ and a relative humidity of 50%.

The homogeneity was verified by the determination of the 12 CBs selected for certification (CBs 28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 163, 170 and 180). The milk powder (3 g) was mixed with 10 mL of water at 40°C and stored for 10 minutes at room temperature. Silica gel (15 g) was added to the wetted material and the resulting mixture was placed on top of a silica gel (30 g) absorption column. This silica was deactivated beforehand to 10% with water. The chlorobiphenyls were eluted with petroleum ether (200 mL). The internal standard, tetrachloronaphthalene, was added and the volume reduced to 2 mL by evaporation for the final determination by GC-ECD using a splitless injector on two fused silica columns. A raw extract was produced to determine the repeatability of the method. A subsample of 20 g milk powder was saponified in an oil bath by refluxing with ethanolic potassium hydroxide (100 mL, 100 g kg⁻¹) for 2 h at 90°C. The cooled mixture was extracted three times in n-hexane (400 mL) and the hexane layer washed with water (100 mL). The organic layer was cleaned-up in seven separate portions of 50 mL on a silica gel column containing 30 g of adsorbent deactivated to 10% with water. The elution of each portion was performed with petroleum ether. The repeatability of the method was assessed by one determination of the PCBs in each of the seven raw extracts and found to be around 2–3% for all compounds, except for CB 52 (7%) due to a very low concentration of this congener. The results of the within and between-ampoule homogeneity study showed that there was a good agreement between the CVs obtained and the material was hence considered to be homogeneous for the compounds of concern [32].

The stability of the material was verified at -20°C, +20°C and +35°C by the determination of the CB congeners over a period of 12 months. In addition, the stability of the dry mass, free fatty acids and fat content was monitored as key indicators of the stability of the matrix. The results showed that the CB congeners were stable at the three temperatures tested, as well as the matrix components.

7.11.3. Certification

The final determination of CBs carried out by all participating laboratories (see section 7.10.7) was based on capillary column gas chromatography with electron capture

detection. In addition, mass spectrometry was used as a complementary technique to confirm the identity of each of the selected CBs in the dried milk powder. The participating laboratories had previously demonstrated that GC-MS was less precise for the determination of these CBs at such levels of concentration so this technique was not used to obtain quantitative data for the certification exercise [32]. High resolution (HR-MS) has proven to achieve a sufficient sensitivity comparable to ECD and has been successfully applied in the certification of CBs in this milk powder material.

Each laboratory used its own optimised procedure for the sample preparation, clean-up, method of injection, calibration and gas chromatographic conditions [33]. Calibrants were obtained as pure crystalline certified materials from BCR [32]; in addition, CBs 105, 128, 149, 156, 163 and 170 were obtained from BCR after characterisation in an independent laboratory of the identity of the compound by elemental analysis, ^1H NMR and melting point determination, and of the purity of the crystalline materials by GC-MS, GC-ECD [32]; these pure compounds were used to prepare calibration solutions for these congeners. Each laboratory prepared separate calibration solutions in iso-octane of the appropriate concentration to calibrate the detector within the approximately linear range. Calibration procedures used by the participants are described in details in the certification report [32].

The choice of internal standards was left to each individual laboratory. However, a series of pure dichlorobenzylalkyl ethers (DCBEs) was made available by the Scottish Office, Agriculture and Fisheries Department (United Kingdom) for use with the calibrants and samples. A list of internal standards used by the participating laboratories is given in the certification report [32].

Before extraction and clean-up the samples were first wetted with an equal amount of bidistilled water at 40°C at room temperature overnight. The samples were then mixed with silica gel and extracted with a mixture of a polar solvent and a non-polar solvent e.g. acetone/hexane, acetone/petroleum ether, methanol/ethyl acetate/hexane, or a non-polar solvent e.g. pentane, hexane, methanol after saponification or oxidation.

Clean-up procedures were applied to remove the bulk of the co-extracted lipid and other organic compounds that would interfere with the chromatographic determination of CBs, either by masking the chromatographic response or by degrading the performance of the column. Different techniques were used, e.g. decomposition with concentrated sulphuric acid (in some cases loaded on silica gel), absorption chromatography (e.g. alumina column), separation with silica gel, Florisil or potassium silicate [32].

Each CB was identified and confirmed using at least two capillary columns coated with a different stationary phase to compare the relative retention times or retention indices of the peaks from calibrants with the peaks from the dried milk powder chromatograms. The GC column conditions are described extensively in the certification report [32].

Recovery experiments were requested to improve the accuracy of the determination. Replicate recovery measurements were carried out by the standard addition of each CB to the dried milk powder matrix. Laboratories whose results were accepted for certification all had recoveries between 80% and 110%.

A number of the congeners, including CB 28, CB 105 and CB 128 are metabolised in the bovine digestive tract. This metabolism alters the relative concentration of the

different CBs in the milk with lower chlorinated congeners, in particular, being present at the ultra-trace level. It is difficult to obtain measurements of sufficient accuracy and precision for certification at concentrations which were encountered in the material and these compounds could hence not be certified; the values are given as indicative in the certification report [32].

There was some disagreement between the data from the different laboratories for CB 101. Consequently, this congener was regarded as 'not quantified'. This was primarily due to the low content of this CB and possible interferences from other materials still present after clean-up.

CB 138 cannot be resolved from CB 163 on most common chromatographic column phases. It requires negative-ion chemical ionisation mass spectrometry to confirm the presence of both congeners and a very polar column to separate them. However, using this type of column, it is possible that other CBs interfere with CB 138 since the retention indices for all congeners on these columns may not be known. Since it has been confirmed that this milk powder contains both CB 138 and CB 163 and that most participating laboratories were unable to make a chromatographic separation between these congeners, the sum of mass fractions of CB 138 + CB 163 were given as indicative values [32]. The separation of CB 138 and CB 163 can be further complicated by CB 158, depending on the capillary column phase used. Some laboratories undertook different separations using alternative GC phases. The indicative values for the separate CBs and different combinations are given in the certification report.

CB 149 was not certified because there was no sufficient agreement between the participating laboratories. This could be due to the potential overlap between CB 118, which closely elutes with CB 149 on some GC phases. Since there was no reasonable explanation for these results, these data were rejected and given only as indicative values [32].

The certified values are given in Table 7.25.

7.11.4. Participating laboratories

The material was prepared by the Milchwirtschaftliche Untersuchungs-und Versuchsanstalt in Kempten (Germany) which also carried out the homogeneity and

TABLE 7.25

CERTIFIED CONTENTS OF CBs IN MILK POWDER CRM 450

Compound	Certified value ($\mu\text{g kg}^{-1}$)	Uncertainty ($\mu\text{g kg}^{-1}$)
CB 52	1.16	0.17
CB 118	3.3	0.4
CB 153	19.0	0.7
CB 156	1.62	0.20
CB 170	4.8	0.6
CB 180	11.0	0.7

stability studies. The following laboratories participated in the certification campaign: Netherlands Institute for Fisheries Research, Ijmuiden (The Netherlands); Swedish Environmental Protection Board, Solna (Sweden); Milchwirtschaftliche Untersuchungs-und Versuchsanstalt, Kempten (Germany); Universität Ulm, Abt. Analytische Chemie (Germany); Agricultural Research Centre, Jokioinen (Finland); Institut Quimic de Sarria, Barcelona (Spain); The Scottish Office Agriculture and Fisheries Department, Aberdeen (United Kingdom); State Institute for Quality Control for Agricultural Products, Wageningen (The Netherlands); Laboratoire Cantonal, Genève (Switzerland); Institute for Marine Research, Bergen (Norway); EC Joint Research Centre, Ispra (Italy); Institut du Génie de l'Environnement, Lausanne (Switzerland); Vrije Universiteit, IVM, Amsterdam (The Netherlands).

7.12. DIOXINS IN MILK

7.12.1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), collectively known as dioxins, comprise a group of, respectively, 75 and 135 individual chemical compounds. These compounds are highly lipophilic and resistant to chemical breakdown, and therefore, they tend to accumulate in the food chain. Many laboratories in Europe are involved in the analysis of environmental and food samples for dioxin compounds, thus creating a requirement for appropriate reference materials. This section describes the certification of five PCDDs and six PCDFs, which are listed below [34]:

D48	2,3,7,8-Tetrachlorodibenzo-p-dioxin
D54	1,2,3,7,8-Pentachlorodibenzo-p-dioxin
D66	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin
D67	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin
D70	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin

F83	2,3,7,8-Tetrachlorodibenzo-furan
F94	1,2,3,7,8-Pentachlorodibenzo-furan
F114	2,3,4,7,8- Pentachlorodibenzo-furan
F118	1,2,3,4,7,8-Hexachlorodibenzo-furan
F121	1,2,3,6,7,8-Hexachlorodibenzo-furan
F130	2,3,4,6,7,8-Hexachlorodibenzo-furan

7.12.2. Production of the material

A quantity (1650 L) of cooled (4°C) cow milk was transported to NIZO in Ede (The Netherlands). The milk was heated to 74°C for 10 seconds, homogenised at 55°C under 200 bar and further cooled to 5°C. The homogenised product was concentrated to 46% (mass fraction) dry matter in four temperature steps from 74°C to 46°C. The concentrate was spray-dried at a temperature of 72°C to give a final water content of about 2%

(mass fraction). The powder was mixed before being placed into 6 bags of 25 kg each and transported to the IRMM in Geel (Belgium).

The 150 kg milk powder was mixed for 2 h using a turbula-mixer before transferring 100 g portions to clean 250 mL brown glass bottles. The bottles were flushed with dry air and closed with aluminium inserts and plastic screw caps. About 1500 bottles were prepared and labelled.

The water mass fraction of the milk powder was determined on 18 samples regularly taken during the filling procedure and was measured by Karl-Fischer titration; the water content was found to be $(2.1 \pm 0.2)\%$ (mass fraction).

The homogeneity was verified by PCDD and PCDF determinations according to the IDF reference method [35,36]. ^{13}C labelled PCDD and PCDF internal standards were added to a known amount of milk powder. After rehomogenization the fat was extracted quantitatively. The native and labelled PCDDs and PCDFs were separated from the fat by gel permeation chromatography and purified using columns of basic alumina and of porous, graphitised carbon. The final determination was carried out by capillary gas chromatography and high-resolution mass spectrometry. Internal quality control samples (blank and fat samples containing known amounts of PCDD and PCDF) and chemical blanks were analysed with the milk powder samples. Because of the very low content of F83, F94 and F124 and the resulting large uncertainty of the measurements, the data of these three congeners could not be used to verify the homogeneity. For the other compounds, the within-bottle homogeneity variances were, as expected, much smaller than the between-bottle homogeneity variances which were, however, in good agreement with the variances of the data obtained in the certification campaign [34]. Therefore, the material was considered to be homogeneous at a level of 5 g and above.

The stability was verified at -20°C , $+20^\circ\text{C}$ and $+37^\circ\text{C}$. No instability could be demonstrated for the PCDD and PCDF congeners at these temperatures over a 12 months period [34].

7.12.3. Certification

Each laboratory was given a free choice of extraction, clean-up and conditions for the final determination. The latter, gas chromatography with high-resolution mass spectrometry, included choice of injection, capillary columns, ion masses monitored etc. [34]. In addition to the CRM, the participants received a calibrant solution containing the PCDDs/PCDFs of interest for this project as a control for their calibration procedure.

The participants were required to use at least one ^{13}C internal standard for each isomeric group of congeners. The internal standards were added before starting the extraction procedure, to either the milk powder or to liquid milk reconstituted from the milk powder as appropriate.

A wide variety of combinations of adsorbents were used for clean-up [34], e.g. open, gravity-fed columns, or flow controlled columns. One laboratory used a dialysis technique to separate PCDDs and PCDFs from the bulk of the fat.

It was required that the final determination should be carried out by capillary gas chromatography with high resolution mass spectrometric detection and that two isotope

peaks should be measured for each analyte. Identification had to be based on comparison of retention times in the samples and the calibration chromatograms and by an agreement of the isotope ratios for PCDDs and PCDFs in the sample extracts with theoretical values. Quantification used relative peak areas of the two major isotope peaks. Two laboratories analysed samples on two GC columns of different polarity to verify the absence of interferences with other compounds; no interferences could be detected. All participants checked the linearity of the system for each of the congeners to be determined, injecting five calibrant solutions, each of a different concentration.

GC conditions are summarised in the certification report [34], as well as the most relevant MS conditions. A wide variety of temperature programmes was employed; in all cases, electron impact ionisation with resolutions above 6000 was used.

Data obtained from seven laboratories out of nine for F124 corresponded to the limits of detection; consequently, this compound could not be certified.

No information on calibrants was available for a series of compounds (D73, D75, F131, F134 and F135) which were hence not certified and given as indicative values only.

Some recommendations could be drawn from the technical discussions, which are summarised in the certification report [34]. In particular, it was stressed that some common extraction procedures may not be suitable for complete extraction of fat and of the analytes from the material. Soxhlet extraction with pure toluene was not found to be adequate; quantitative extraction can be achieved by Soxhlet extraction with ethanol/toluene, or by slurring the milk powder with water, mixing and drying with sodium sulphate and silica, followed by Soxhlet extraction with hexane/acetone. Procedures based on the AOAC protocols applied on reconstituted milk can also be recommended; all these procedures are based on liquid/liquid extraction and therefore emulsion is possible.

The certified values are given in Table 7.26.

TABLE 7.26

CERTIFIED CONTENTS OF DIOXINS IN MILK POWDER CRM 607

Compound	Certified value (ng kg ⁻¹)	Uncertainty (ng kg ⁻¹)
D48 — 2,3,7,8-TCDD	0.25	0.03
D64 — 1,2,3,7,8-PeCDD	0.79	0.04
D66 — 1,2,3,4,7,8-HxCDD	0.42	0.07
D67 — 1,2,3,6,7,8-HxCDD	0.98	0.11
D70 — 1,2,3,7,8,9-HxCDD	0.34	0.05
F83 — 2,3,7,8-TCDF	0.05	0.03
F94 — 1,2,3,7,8-PeCDF	0.054	0.013
F114 — 2,3,4,7,8-PeCDF	1.81	0.13
F118 — 1,2,3,4,7,8-HxCDF	0.94	0.04
F121 — 1,2,3,6,7,8-HxCDF	1.01	0.09
F130 — 2,3,4,6,7,8-HxCDF	1.07	0.05

7.12.4. Participating laboratories

The material was prepared by the Netherlands Institute for Dairy Research (NIZO) in Ede (The Netherlands), the Rijks-Kwaliteitinstituut voor Land- en Tuinbouwprod. (RIKILT-DLO) in Wageningen (The Netherlands) which also carried out the homogeneity and stability studies, and the Institute for Reference Materials and Measurements in Geel (Belgium). The following laboratories participated in the certification campaign: Chemische Landesuntersuchungsanstalt, Freiburg (Germany); Centre d'Analyse et de Recherche sur les Substances Organiques, Lyon (France); ERGO-Forschungsgesellschaft mbH, Hamburg (Germany); National Public Health Institute, Kuopio (Finland); Rijksinstituut voor Volksgezondheid en Milieuhygiene (RIVM), Bilthoven (The Netherlands); RIKILT-DLO, Wageningen (The Netherlands); Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol (Belgium); University of Umeå, Institute for Environmental Chemistry, Umeå (Sweden).

7.13. MICROBIOLOGICAL PARAMETERS IN MILK

7.13.1. Introduction

Quality control in microbiological testing and research has become of growing interest in the past 5–10 years. For this purpose, many procedures for microbiological analysis have achieved the status of national or even international standard method. It should be stressed that the use of standard methods does not always guarantee a good day-to-day repeatability in one laboratory or comparable results in different laboratories. For internal quality control, many laboratories have prepared their own reference materials. However, the preparation of microbiological reference materials with assigned values is very time consuming and stabilising living organisms is mostly very difficult. Furthermore, a comparison of results of different laboratories with their individual home-made reference materials is hardly possible. The BCR has hence stimulated the development of microbiological (certified) reference materials. These materials consist of gelatin capsules filled with milk powder artificially contaminated with a bacterial test strain. The first certification study focused on milk CRM containing *Salmonella typhimurium* [37] whereas the second certification dealt with *Enterococcus faecium* [38].

7.13.2. Production of the reference materials

7.13.2.1. Milk containing *Salmonella*

For the development of the reference material containing *Salmonella* a strain of the serotype *typhimurium* was chosen as a common serotype found in food and feed and frequently involved in foodborne infections. The strain was identified and confirmed by three independent laboratories, following testing methods fully described elsewhere [37].

Salmonella typhimurium (ALM 40) was streaked for purity onto a sheepblood agar dish and incubated for 24 h at $(37 \pm 1)^\circ\text{C}$. A single colony was used for inoculation of

2 L heart infusion broth which was subsequently incubated at 37°C for ca. 24 h. After incubation, the culture was divided over 250 mL centrifuge tubes and centrifuged for 15 min at 5000 rpm. The supernatant was discarded and the pellet was re-suspended in 2 mL peptone saline solution and added to pasteurised full fat milk (evaporated to a dry mass concentration of 450 g L⁻¹). The milk was spray dried after homogenisation using a pilot plant spray dryer at an inlet temperature of ca. 140°C and an outlet temperature of ca. 80°C. The resulting highly contaminated milk powder had a number content of colony forming particles of ca. 10⁴ g⁻¹, contained fat at a mass fraction of 0.25 and was stored at 5°C.

To obtain the desired number content of colony forming particles, 30.8 g of the highly contaminated milk powder was mixed with 2.5 kg of sterile milk powder. The powder was filled in plastic bags placed in metal drums and sterilised by gamma irradiation with a dose of 10 kGy. The irradiated powder was stored at room temperature. The mixing of the highly contaminated milk powder with the skim milk powder was carried out in a 17 L stainless steel drum with a Turbula mixing apparatus for 4 h at room temperature. After mixing, the drum was stored at 5°C in a refrigerator with silicagel drying of the air.

The mixed powder was filled in gelatin capsules in a laminar air flow cabinet with 17 g powder for 60 capsules using an aluminium filling apparatus sterilised for 15 min at 121°C. The empty capsules were sterilised by gamma irradiation with a dose of 10 kGy. The average mass of a filled capsule, including the capsule itself, was (0.359 ± 0.009) g. The number of colony forming particles of *Salmonella typhimurium* in one capsule was determined to be 5.04. A total of around 8.8000 capsules was produced.

The homogeneity and stability of the reference material were verified on the basis of enumeration results as described in the certification report [37]. The material was considered to be homogeneous as verified by the analysis of 250 capsules. The stability was studied at 5°C and -20°C over 90 weeks. Statistical analysis showed that the strain content did not decrease significantly at -20°C over the period tested; storage at 5°C, however, resulted in a significant decrease of 0.35% per week. An additional stability study was carried out at 22°C, 30°C and 37°C for a 4-week storage period; at 22°C no significant decrease was observed whereas at 30°C a significant decrease of 3% per day was shown. At 37°C, a significant decrease occurs after some days of storage [37].

7.13.2.2. Milk containing *Enterococcus*

The test strain used for the preparation of the batch of reference material is *Enterococcus faecium*, which has been isolated from water. The identification was based on (1) biochemical identification, (2) identification with the API-20-STREP method, and (3) serological testing for group D antigen, which confirmed the strain of *Enterococcus faecium*. These methods are described in details elsewhere [38].

Commercial sterile evaporated milk was used for the preparation of the reference material, with a dry mass concentration of 200–300 g L⁻¹ and a fat mass concentration of 40 g L⁻¹. Pink gelatin capsules were used, gamma irradiated with a dose of 10 kGy prior to use. A test strain *Enterococcus faecium* was cultured in 100 mL THB at 37°C for 48 h, while shaking at 100 rpm. Next the culture was centrifuged for 20 min at 2000

G, the supernatant was removed and the pellet was resuspended in 3 L sterilised evaporated milk and thoroughly mixed on a magnetic stirring device. The mean number concentration of colony forming particles of *Enterococcus faecium* in the milk suspension was measured by the spread plate technique using tenfold dilutions on THA. The milk suspension was spray-dried at an inlet temperature of ca. 190°C and an outlet temperature of 70–75°C. The resulting highly contaminated milk powder was stored at 5°C. The mean number content of colony forming particles of *Enterococcus faecium* was (roughly) determined one month after spray-drying. For this purpose, 1 g highly contaminated milk was mixed, in one step, with 100 g sterile milk powder (previously gamma irradiated at a dose of 10 kGy) for 8 h at room temperature. The powders were mixed in a glass vessel with a nominal volume of 1 L in the mixing apparatus. Next gelatin capsules were filled with 0.214 g mixed powder per capsule. Ten capsules were reconstituted in 10 mL peptane saline solution according to the procedure described in the certification report [38]. Tenfold dilutions in peptane saline solution were performed and enumerations were carried out by means of membrane filtration. The plates were incubated at 37°C for 48 h. The strain survived the spray-drying process very well; however, it was sub-lethally injured which was demonstrated by the fact that the recovery of a non-selective medium (THA) was better than on a selective medium (KFA) [38].

The mixing procedure for the final batch was carried out in several steps. A preliminary batch of 60 capsules was filled with 0.258 g each of mixed milk powder and twenty of these capsules were used to determine the number content of colony-forming particles; the criteria for acceptance of this test batch were met and hence the filling of the capsules for the preparation of the final batch was started. For the filling of the capsules an apparatus was used in which 60 capsules could be filled in one step. In total 7380 capsules (123 times 60 capsules) were filled in four days by one person. For every filling, two capsules were used to determine the number content of colony forming particles; hence 246 capsules were measured in 5 days by 2 persons. No large deviations were found between the mean number content of colony forming particles of the different filling steps. Some problems with the reconstitution procedure resulted in some high values of the within capsule variation; these problems were solved by improvement of the reconstitution procedure. It was concluded that the final batch was prepared in the best possible way.

The variation in the number content of colony-forming particles between samples in one reconstituted capsule (T_1) and between samples of different capsules of one batch (T_2) were tested separately. Although the T_1 -value exceeded the critical values twice, the batch was still considered as being of good quality because the deviations of the critical value were small and considered to be of random nature. An additional homogeneity verification was performed using two alternative methods as described in the certification report [38].

The stability of the material was verified at –20°C over 8 months by enumerating the number content of colony forming particles by means of membrane filtration as described elsewhere [38]. An additional stability study was carried out at 22°C, 30°C and 37°C over 30 days storage to simulate possible changes during transport conditions. No significant decrease was observed at –20°C over 8 months; a preliminary batch has been shown to be stable over 14 months [38]. With respect to higher temperatures, no significant

changes were noticed at 22°C over 31 days; storage of the materials at 30°C resulted in a slow decrease and at 37°C in an immediate decrease of the number content of colony forming particles. Additional details on the methods used to test the homogeneity and stability and all individual data are described in the certification report [38].

7.13.3. Certification

7.13.3.1. Milk containing *Salmonella*

For the certification of the *Salmonella* reference material, two different procedures were used. The first procedure determined the number of colony forming particles in one capsule whereas the second procedure determined the presence or absence of *Salmonella* in one capsule. Both procedures were carried out according to a detailed protocol and Standard Operating Procedures described elsewhere [37].

For the enumeration procedure, each laboratory received 60 capsules and a maximum number of 50 enumerations was requested. The extra capsules were supplied to replace the capsules that were not acceptably dissolved. The capsules were dissolved in 2 rounds each of 30 capsules. For each series also a control was tested, consisting of a Petri-dish containing peptone saline to which no capsule was added. The procedure can be summarised as follows: (1) dissolution of a capsule in 5 mL peptone saline solution in a Petri-dish for (45 ± 5) min in an incubator at $(38.0 \pm 0.5)^\circ\text{C}$ while shaking at ca. 100 rpm; (2) repair of *Salmonella* in Plate Count Agar (incubation time (4 ± 1) h at $(37 \pm 1)^\circ\text{C}$); (3) selective growth of *Salmonella* by adding Violet Red Bile Glucose agar on top of the Plate Count Agar (incubation time (20 ± 2) h at $(37 \pm 1)^\circ\text{C}$); and (4) counting of typical colonies and confirmation of ca. 20 typical colonies (by means of biochemical and/or serological tests of own choice). In earlier pilot studies, the dissolution step has been shown to be the most critical step in this procedure. Therefore, much attention has been paid to this step. Seven laboratories, which used a biochemical confirmation or a biochemical and a serological confirmation, confirmed all colonies tested as *Salmonella*. Four laboratories, which used only serological confirmation, confirmed 86% of the colonies tested; the remaining 14% were all confirmed as *Salmonella* by means of an additional confirmation.

The presence/absence procedure was based on the ISO method for the detection of *Salmonella* (ISO 6579) which is summarised as follows: (1) pre-enrichment in Buffered Peptone water (incubation time (18 ± 2) h at $(37 \pm 1)^\circ\text{C}$); and (2) selective enrichment in broth of own choice (incubation time and temperature according to own procedure). The detailed procedure is described elsewhere [37]. Each laboratory determined the presence or absence of *Salmonella* in 50 capsules. Four of these individually identified capsules were negative control capsules. The numbers of these capsules were unknown to the laboratories at the time of analysis. For the presence/absence procedure, all capsules showing typical colonies on the isolation agar were subjected to a confirmation for *Salmonella*. At least two colonies per capsule were used for this confirmation. All colonies (>1000 colonies) tested by the laboratories gave a positive *Salmonella* identification. The type of confirmation test used is described elsewhere [37].

The data submitted by the laboratories were in good agreement, with the exception

of one laboratory which set was not used as a result of an error of the incubation period. Statistical tests were especially adapted for analysing the counts reported by the laboratories [37]. In particular, the variation between capsules was calculated by means of the T_2 test which did not allow to detect laboratories with exceptional values. In addition, none of the laboratories found a higher value than the critical (calculated) level. Additional details on the statistical tests and method comparisons are given in the certification report [37].

The certified values are given in the Table 7.27.

Special precautions to maintain the integrity of the reference material are described in the certification report [37]; they include the storage at $(-20 \pm 5)^\circ\text{C}$, the handling procedure (e.g. equilibration, use of sterile forceps, etc.), and advice on the detection procedure for *Salmonella*.

7.13.3.2. Milk containing *Enterococcus*

Each laboratory that participated in the certification campaign received, by courier service, 15 capsules of candidate reference material containing *Enterococcus faecium* and a set of detailed instructions [38]. The laboratories were requested to reconstitute each capsule in 10 mL solution and to perform duplicate enumerations of the number concentrations of *Enterococcus faecium* (each determination with 1 mL of the

TABLE 7.27
CERTIFIED VALUES OF *SALMONELLA* IN MILK POWDER CRM 507

CRM 507			
Number fraction of negative capsules and number of colony forming particles of <i>Salmonella typhimurium</i> in artificially contaminated milk powder			
Quantity (test procedure)	Certified value	Confidence limit(s)	Sets of accepted results
number of <i>Salmonella</i> colony forming particles in one capsule (enumeration procedure)	5.9	5.3 – 6.4 ^a	10 ^c
fraction of capsules in which no <i>Salmonella</i> could be detected (enumeration procedure)	0.61%	0 – 1.6% ^b	10 ^d
fraction of capsules in which no <i>Salmonella</i> could be detected (presence/absence procedure)	2.7%	0 – 4.4% ^b	9 ^e

- a two sided 95% confidence interval
- b one sided 95% confidence upper limit
- c based on the results of 485 capsules
- d based on the results of 492 capsules
- e based on the results of 414 capsules

reconstituted capsule). The procedures were to be carried out according to the two ISO method prescriptions ISO 6222, 1988 and ISO 7899/2, 1994 which are detailed elsewhere [38]. ISO 6222 describes the enumeration of viable micro-organisms in water; for this enumeration, a pour plate technique is used with the non-selective medium Yeast Extract Agar (YA). ISO 7899/2 describes the detection and enumeration of faecal streptococci in water; for this enumeration, a membrane filtration technique is used with two selective media (both used in the certification): Kenner Fecal Agar (KFA) and membrane *Enterococcus* Agar (m-EA). Details on the procedures and on the most important criteria for proper performance are given in the certification report [38].

The participating laboratories had to perform their measurements under strict quality control (e.g. control of the media, of the membrane filters, control and/or adjustment of the incubator and or the waterbath, blank controls, etc.). At the technical meeting, all sources of possible errors were discussed and doubtful results were discarded. Typical errors were related to poor dissolution of the capsules and pH higher than 7.4 in the KFA-medium (affecting bacterial growth). The certified values are given in Table 7.28.

Similarly to CRM 507, special precautions to maintain the integrity of the reference material are described in the certification report [38].

7.13.4. Participating laboratories

The preparation of the CRM 507 was carried out by the Laboratory of Water and Food Microbiology of the National Institute of Public Health and Environmental Protection (RIVM) in Bilthoven (The Netherlands) which also performed the

TABLE 7.28

CERTIFIED VALUES OF *ENTEROCOCCUS FAECIUM* IN MILK POWDER CRM 506.

The units are given in number of colony forming particles per ml reconstituted milk

CRM 506 Number of colony forming particles of <i>Enterococcus faecium</i> in artificially contaminated milk powder			
Method	Certified value ¹	95% confidence limits ²	Sets of accepted results ³
ISO 7899/2, 1984 KFA	79	70–88	7
ISO 7899/2, 1984 m-EA	76	70–83	7
ISO 6222, 1988 YA	110	100–121	8

1 Geometric mean of n accepted sets of data

2 95% confidence limits belonging to the certified values of the specified method

3 Total number of individual results was 206 for KFA, 197 for m-EA and 220 for YA

identification and the characterisation of the test strain and verified its homogeneity and stability. The identification of the strains was performed by the Division of Enteric Pathogens of the Central Public Health Laboratory in London (United Kingdom), the International *Salmonella* Centre of the Institut Pasteur in Paris (France). The following laboratories participated in the certification campaign: Campden Food & Drink Research Association, Chipping Campden (United Kingdom); Central Institute for Nutritional Research, Zeist (The Netherlands); Institut für Lebensmittelhygiene, Universität Berlin (Germany); Istituto di Tecnologie Alimentari, Udine (Italy); Laboratório Nacional de Engenharia e Tecnologia Ambiental, Lisbon (Portugal); Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge (United Kingdom); National Institute of Public Health and Environmental Protection (RIVM), Bilthoven (The Netherlands); State Institute for Inspection of Agricultural Products, Wageningen (The Netherlands); Rijkszuivelstation, Melle (Belgium); Istituto di Malattie Infettive, Profilassi e Polizia Veterinaria, Bologna (Italy); Universität Bonn (Germany).

The preparation of the CRM 506 was carried out by the Laboratory of Water and Food Microbiology of the National Institute of Public Health and Environmental Protection (RIVM) in Bilthoven (The Netherlands) which also performed the identification of the test strain in collaboration with the Faculty of the Veterinary Medicine of the University of Gent (Belgium). The following laboratories participated in the certification: Abteilung und Umwelthygiene, Universität Tübingen (Germany); Instituto de Salud Publica de Navarra, Pamplona (Spain); Institut Pasteur, Laboratoire de Microbiologie, Lille (France); Centre de Recherche Lyonnaise des Eaux/Dumez, Le Pecq (France); Athens School of Hygiene, Bacteriology Department, Athens (Greece); University College, Food Hygiene Laboratory, Dublin (Ireland); National Institute of Public Health and Environmental Protection (RIVM), Bilthoven (The Netherlands); Provincial Waterworks North-Holland, Haarlem (The Netherlands); Instituto Nacional de Saude Dr Ricardo Jorge, Lisbon (Portugal); General Hospital, Regional Public Health Laboratory, Newcastle (United Kingdom); Water Research Centre, Medmenham (United Kingdom).

7.14. TRACE ELEMENTS IN HUMAN HAIR

7.14.1. Introduction

Some authors have stated that human hair can be employed as an index for an excess, or a deficiency, of specific nutrients in the diet or as an index of absorption of contaminants from the environment [39]. Analysis of hair is considered to give an indication of the integrated dose of elements ingested by a person over some months. In addition, it is easier to collect hair than blood or urine. Therefore, analysis of human hair is performed by a number of organisations to monitor the level of exposure to heavy metals of a population or an individual [40]. However, this monitoring is often hampered by an insufficient reliability of the measurements. The BCR has thus decided to provide laboratories with a mean of checking their analytical performance by

producing a human hair reference material, which was certified for a range of trace elements [41].

7.14.2. Production of the material

Over 6 kg of hair material resembling an average population in Northern Italy has been collected in the late seventies; after washing with water and drying, it has been ground in a tungsten carbide mill under liquid nitrogen. The resulting powder passed a sieve with 80 μm apertures. A careful examination of the material under a magnifying-glass indicated that there were no pieces of hair of longer size which had passed the sieve longitudinally. The material was homogenised under argon in a closed mixing drum with PTFE lining for two weeks; it was bottled in glass bottles provided with screw caps and plastic inserts (approx. 10 g per bottle) after addition of a PTFE-ball to facilitate later re-homogenisation.

The homogeneity was verified by INAA determinations of Al, As, Br, Cl, Co, Cr, Fe, Hg, K, Mn, Mo, Se and Zn on intakes of 100 mg. The results showed that the material is homogeneous at this level of intake [41].

The stability of the material was tested at -20°C , $+20^{\circ}\text{C}$ and $+40^{\circ}\text{C}$ over a period of 12 months by determining Br, Na, Se and Zn at regular intervals over the storage period. Analyses were carried out by INAA. No instability could be demonstrated and the material was hence kept stored at ambient temperature [41,42].

7.14.3. Certification

Eighteen laboratories from nine European countries participated in the certification (see section 7.7.4). Table 7.29 summarises the different techniques of final determination used; pretreatment techniques were based on digestion with combination of acids in pressurised or atmospheric mode, programmed dry ashing, combustion and irradiation with thermal neutrons. A detailed description of the methods is given in the certification report [41].

The presence of systematic errors was suspected when discussing the results of As

TABLE 7.29

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN HUMAN HAIR CRM 397

Elements	Techniques of final determination
Cd	DPASV, ETAAS, FAAS, INAA, MS, ZETAAS
Hg	CVAAS, CVAFS, HICP, INAA
Pb	DPASV, FAAS, ETAAS, ICP-AES, MS, ZETAAS
Se	HGAAS, RNAA, ZETAAS
Zn	DPASV, FAAS, ICP-AES, INAA, MS

TABLE 7.30

CERTIFIED CONTENTS OF TRACE ELEMENTS IN HUMAN HAIR CRM 397

Element	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)
Cd	0.521	0.023
Hg	12.3	0.6
Pb	33.0	1.1
Se	2.00	0.07
Zn	199	5

due to a large spread of results and internal inconsistency of the methods which hampered certification of this element.

Human hair is difficult to digest completely due to high contents of sulphur and silica. It was strongly recommended in the certification to perform pressurised digestions with oxidising acids, followed by repeated additions of HF/HNO₃ and evaporation to dryness.

The certified values are listed in Table 7.30. Indicative values are published elsewhere [41,42].

The comparison of methods for some elements did not allow to detect any bias due to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 7.31).

TABLE 7.31

COMPARISON OF METHODS FOR Cu, Hg, Pb, Se AND Zn IN CRM 397

Element	Techn. of final determination	CV % between means of lab. with the same technique	Nr of sets or results	CV % between means of diff. techniques
Cu	ETAAS	9.7	5	6.0
	DPASV	7.6	4	
Hg	INAA	5.3	4	2.9
	CVAAS	7.2	5	
Pb	FAAS	6.5	4	3.0
	ETAAS	10.8	3	
	ICP-AES	2.7	4	
	DPASV	6.4	4	
Se	RNAAS	5.3	3	0.3
	HGAAS	4.2	3	
Zn	INAA	2.6	3	1.1
	FAAS	2.3	3	
	ICP-AES	7.0	4	

7.14.4. Participating laboratories

The material was prepared by the EC Joint Research Centre of Ispra (Italy). The homogeneity and stability studies were carried out at the Energieonderzoek Centrum Nederland in Petten (The Netherlands).

The following laboratories participated in the certification: Aristotle University, Lab. Analytical Chemistry, Thessaloniki (Greece); Coronel Laboratory, University of Amsterdam (The Netherlands); Danish Isotope Centre, Copenhagen (Denmark); Danish Technological Institute, Taastrup (Denmark); Energieonderzoek centrum Nederland, Petten (The Netherlands); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Istituto Superiore di Sanità, Roma (Italy); Institut für Spektrochemie und angewandte Spektroskopie, Dortmund (Germany); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Kernforschungsanlage, Jülich (Germany); Laboratory of the Government Chemist, Teddington (United Kingdom); Presidio Multizonale di Prevenzione, Sezione Chimica, Venezia (Italy); Risø National Laboratory, Roskilde (Denmark); Service Central d'Analyse, CNRS, Vernaison (France); Universidad Complutense, Facultad de Química, Madrid (Spain); Università degli Studi di Pavia, Centro di Radiochimica Generale, Pavia (Italy).

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Chapter 8

CRMs for water analysis

8.1. MAJOR ELEMENTS IN FRESHWATER

8.1.1. Introduction

During recent years a number of Directives have been issued, concerned with the quality of several types of water, e.g. drinking water (75/449/CEE, 79/869/CEE, 80/778/CEE). They prescribe the determination of a range of major elements such as Al, Ca, Cl, Fe, Mg, Mn, P, K, Na and S. The high number of determinations performed per year by control laboratories for water quality assessment imply that suitable CRMs should be available for quality control. This section describes the certification of two artificial freshwater reference materials, one with a low mineral content (CRM 398) and one with a higher mineral content (CRM 399) [1].

8.1.2. Production of the materials

A 260 L polypropylene tank was used for the homogenisation; this tank was thoroughly cleaned by filling it close to the brim with filtered acid tap water, acidified with nitric acid to a final concentration of 0.1 mol L^{-1} , which was circulated in the tank by pumping continuously for several days. The tank was rinsed with deionised water and refilled with 0.1 mol L^{-1} nitric acid (subboiling quality) deionised water. After soaking for one week the tank was emptied and rinsed again with deionised water. Immediately before the bottling run of each CRM the tank was cleaned again using acidified deionised water.

Polypropylene bottles were studied for possible contamination or losses through evaporation. The test consisted of filling ten bottles with freshly prepared 0.1 mol L^{-1} nitric acid, screwing on the cap and leaving the bottle at room temperature for a period of 1–7 days, before analysing the solutions for each of the 10 determinands. On the day of analysis a fresh batch of 0.1 mol L^{-1} nitric acid was prepared and the solutions from the bottles analysed in the same run with the fresh solution as references. The results did not reveal any significant contamination with the bottles selected. The bottles were carefully cleaned by soaking for 2 h in a diluted detergent solution and rinsed six times inside and outside with deionised water. The bottle caps were treated in a similar way.

For each of the reference materials 1000 bottles were conditioned for 7 days by leaving them in contact (alternating top/bottom positions) with the solution they would contain. For the higher level of mineral matter there was no detectable change of analyte content in 10 bottles tested. For the solution with lower levels, however, the conditioning had to be repeated for 7 additional days [2].

The materials were prepared from silica free deionised water (0.1 mol L^{-1}) to which the compounds of interest were added in the form of acidic aqueous solutions. Sulphate, phosphate and chloride were added as ammonium salts, Al, Ca, Fe, K, Mg and Na as nitrates whereas the solution of Mn was obtained by dissolution of the pure metal in nitric acid. The concentrations expected upon spiking are given in Table 8.1.

Homogenisation was carried out in the polypropylene tank covered with a close fitting polyethylene lid. A centrifugal pump connected to the tank with polyethylene piping ensured constant recirculation of the solution. The pump had no metal parts in contact with the water. An additional cover of heavy gauge polyethylene was fitted over the tank and the pipework to prevent ingress of dust. It was established by separate experiments that mixing for 15 min was sufficient to achieve a good sample homogenisation [2].

The analytical methods used for testing the between-bottle homogeneity were visible light or UV spectrophotometry (Fe, Cl and P), ETAAS (Al and Mn), FAES (K and Na), FAAS (Ca and Mg) and flow injection turbidimetry measurement (S as sulphate). The results did not reveal any significant between-bottle variability and it was concluded that both batches were homogeneous.

The stability was verified by determining the various elements of concern in samples stored at ambient temperature over 12 months. No instability could be demonstrated and the materials were considered suitable for certification.

8.1.3. Certification

The techniques used by the participating laboratories (see section 8.1.4) are summarised in Table 8.2. Pretreatment techniques included dilution, addition of HF (for Al complexation), irradiation with thermal neutrons (for NAA), addition of internal standards (for ICPMS), addition of buffer (for titration) etc.

The relatively high standard deviation of the NAA-techniques for Cl could be

TABLE 8.1

CONCENTRATIONS EXPECTED UPON SPIKING (ADAPTED FROM [2])

Element	CRM 398	CRM 399	Units
Al	30	200	$\mu\text{g kg}^{-1}$
Ca	30	80	mg kg^{-1}
Cl	10	50	mg kg^{-1}
Fe	30	200	$\mu\text{g kg}^{-1}$
Mg	5	15	mg kg^{-1}
Mn	29.9	199.2	$\mu\text{g kg}^{-1}$
K	1.0	3.0	mg kg^{-1}
Na	5	30	mg kg^{-1}
P	0.1	1.0	mg kg^{-1}
S	3.3	8.3	mg kg^{-1}

TABLE 8.2

SUMMARY OF THE TECHNIQUES OF FINAL DETERMINATION (CRMS 398/399)

Element	Techniques
Al	DCP-AES, ETAAS, ICP-AES, ICPMS, INAA, ZETAAS
Ca	DCP-AES, FAAS, ICP-AES, ICPMS, TITR
Cl	CSV, ICPMS, INAA, RNAA, TITR
Fe	DPP, ETAAS, ICP-AES
Mg	DCP-AES, FAAS, ICP-AES, ICPMS, IDMS
Mn	ETAAS, ICP-AES, ICPMS, INAA
P	DPP, ICP-AES, SPEC
K	DCP-AES, FAAS, FAES, ICP-AES
Na	DCP-AES, FAAS, FAES, ICP-AES, ICPMS, INAA
S	IC, ICP-AES, IDMS

explained from counting statistics. There was no reason to suspect poor accuracy of the results.

Nephelometry gave rather large standard deviations for S, which was considered to be inherent to the technique.

A spread in the results of P in CRM 398 and S in CRM 399 was attributed to the presence of unexplained systematic errors; the contents of these elements could not be certified in the respective CRMs.

The certified values are listed in Table 8.3. Indicative values for P (CRM 398) and S (CRM 399) are given in the certification report [1].

The comparison of methods for some elements showed that no particular bias could be attributed to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 8.4)

8.1.4. Participating laboratories

The preparation of the reference materials, as well as the homogeneity and stability studies, were performed by the Anglian Water Authority in Cambridge (United Kingdom). The analyses for certification were carried out by the following laboratories: Energieonderzoek Centrum Nederland, Petten (The Netherlands); Fondazione Clinica del Lavoro, Pavia (Italy); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Istituto Italiano di Idrobiologia, CNR, Pallanza (Italy); Istituto di Ricerca sulle Acque, CNR, Brugherio (Italy); KIWA N.V., Nieuwegein (The Netherlands); Ministère des Affaires Economiques, Brussels (Belgium); Portsmouth Polytechnic, Dept. of Chemistry (United Kingdom); Risø National Laboratory, Roskilde (Denmark); Service Central d'Analyse, CNRS, Vernaison (France); Universidad Complutense, Depto. de Química Analítica, Madrid (Spain); Universiteit Gent, Instituut voor Nucleaire Wetenschappen, Gent (Belgium); Water Quality Institute, Hørsholm (Denmark).

TABLE 8.3

CERTIFIED VALUES FOR CRMS 398 AND 399

p: sets of results (each of at least 5 replicates)

CRM 398	Certified value	Uncertainty	Units
Al	36.3	4.3	$\mu\text{g kg}^{-1}$
Ca	30.0	0.7	mg kg^{-1}
Cl	10.3	0.4	mg kg^{-1}
Fe	29.3	1.4	$\mu\text{g kg}^{-1}$
Mg	5.03	0.06	mg kg^{-1}
Mn	29.8	0.3	$\mu\text{g kg}^{-1}$
K	1.03	0.04	mg kg^{-1}
Na	5.07	0.08	mg kg^{-1}
S	3.39	0.14	mg kg^{-1}

CRM 399	Certified value	Uncertainty	Units
Al	207	9	$\mu\text{g kg}^{-1}$
Ca	79.2	0.9	mg kg^{-1}
Cl	50.5	0.9	mg kg^{-1}
Fe	202	3	$\mu\text{g kg}^{-1}$
Mg	15.1	0.2	mg kg^{-1}
Mn	199	3	$\mu\text{g kg}^{-1}$
K	2.99	0.12	mg kg^{-1}
Na	30.4	0.7	mg kg^{-1}
P	1.01	0.03	mg kg^{-1}

8.2. NITRATE IN FRESHWATER**8.2.1. Introduction**

While the amounts of nitrate could be certified in rainwater CRMs [3], their certification in freshwater CRMs was not possible owing to the stabilisation procedure used (involving an addition of nitric acid) [2]. Consequently, it was decided to organize a separate interlaboratory study of which the final aim was to produce artificial freshwater CRMs certified for their contents of nitrate, one with a low nitrate content (CRM 479) and one with a high nitrate content (CRM 480) [4,5]. These CRMs correspond to values below and slightly above the maximum permissible nitrate content (approx. 50 mg L^{-1}) mentioned in the Drinking Water Directive.

8.2.2. Feasibility study

A feasibility study was carried out to investigate the optimal conditions for the preparation of the candidate CRMs of artificial freshwater to be certified for their

TABLE 8.4

RESULTS OF THE EVALUATION OF CONSISTENCY OF THE METHODS USED FOR CRM 398

Compound	Techn. of final determination	CV (%) between means of lab. with the same technique	Nr of sets of results	CV (%) between means of diff. techniques
CRM 398				
Ca	FAAS	4.8	5	0.3
	ICP-AES	1.5	4	
Fe	ETAAS	6.4	5	4.4
	ICP-AES	5.8	3	
Mg	FAAS	1.3	4	0.5
	ICP-AES	1.2	4	
Mn	ETAAS	1.1	3	0.5
	ICP-AES	0.6	3	
Na	FAAS	3.2	3	0.08
	ICP-AES	1.4	3	
CRM 399				
Ca	FAAS	1.4	3	0.6
	ICP-AES	1.1	3	
Mg	FAAS	2.1	4	1.0
	ICP-AES	0.7	3	
P	SPEC	2.2	6	0.3
	ICP-AES	2.8	3	

nitrate contents [5]. Solutions were prepared at three levels of concentrations, respectively 0.5, 8 and 53 mg L⁻¹, and their stability was verified at +20°C and 40°C over a period of six months.

Possible procedures for achieving a good stability of natural freshwater samples were discussed in a preparatory meeting with the participants in the project. Freezing was not considered to be a suitable procedure for the long-term storage of CRMs of natural water as it may lead to irreversible physico-chemical changes (e.g. formation of insoluble Ca-salts) and would necessitate the use of special containers and ways of dispatching. Freeze-drying would also be susceptible to physico-chemical difficulties. Consequently, in view of the risks of instability of natural waters and of the high variability in composition, it was decided to prepare artificial solutions containing known concentrations of nitrate and other major constituents, if necessary with addition of stabilising agents.

The pH of the solution was considered to be critical for the stability and subject to changes when the sample is in contact with CO₂ (e.g. during bottling). The evaporation of CO₂ as well as the precipitation of CaCO₃ were considered to be possible sources of inhomogeneity and instability. Problems were considered to be less if samples could

be equilibrated with air and if a carbonate buffer would be added in order to avoid major changes. On the basis of the chosen procedure, Na_2CO_3 was added up to a concentration of 530 mg kg^{-1} to maintain the pH against air; the resulting pH ranged between 9 and 9.5 which is higher than the pH observed in natural water. The pH was therefore lowered by adding acid (0.5 mL of $\text{HCl } 10 \text{ mol kg}^{-1}$ in a litre of solution) in order to achieve a pH value of 6.8. Implicitly, Ca could be present to such an extent only that precipitation would not occur and all other ions could be added with the exception of e.g. phosphate and ammonium that might provoke microbiological growth.

The addition of UV-absorbing organic matter was also discussed to match the presence of humic acids; as it was difficult to prepare water samples containing humic acids, the use of lauryl sulphate having a wide absorption range in UV and known to be stable at concentrations in the range $1\text{--}3 \text{ mg L}^{-1}$ was recommended; 2 mg L^{-1} of lauryl sulphate was therefore added to the samples. Finally, phenyl-mercury acetate ($\text{C}_8\text{H}_8\text{HgO}_2$) was added to avoid the development of moulds. Different concentrations were studied and undesirable white precipitate was observed in the samples for concentrations of 100 mg L^{-1} of phenyl-mercury acetate as well as deleterious effects on the redox copperized Cd column at the determination step in the photometric method based on reduction to nitrite; this effect was not noticeable at the 10 mg L^{-1} level which was therefore used for the sample preparation. The levels of nitrate concentrations were chosen to be in the range of levels found in the environment, considering the maximum value specified in the EC Directive (50 mg L^{-1}). For the first interlaboratory study, the participants recommended to prepare samples with concentrations of ca. 1, 10 and 50 mg L^{-1} (respectively solutions A, B and C). The concentrations of the other compounds were $\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$ (91.7 mg L^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (123 mg L^{-1}) and Na_2CO_3 (530 mg L^{-1}) to simulate the hardness of a natural water. Nitrates were added in the form of high purity salts (KNO_3) dissolved in Milli-Q water. The water was boiled, homogenised by mechanical shaking using a magnetic stirrer and filtered with sterile filters to $0.2 \mu\text{m}$. The different compounds were added by pumping.

In order to verify the long-term stability of the samples upon storage, two types of ampoules of 200 mL were selected, in white and brown glass, respectively. The short-term stability (3 months) of the three sets of samples was verified at $+20$ and $+40^\circ\text{C}$. The homogeneity was verified prior to the stability experiments by performing five measurements in fifteen ampoules (for each of the three concentrations considered) randomly selected during the filling procedure. No instability was demonstrated over a period of three months for the three sets of solutions, both in white and brown glass ampoules. Consequently, the white glass ampoules were selected for the storage of the solutions and a temperature of $+20^\circ\text{C}$ was adopted for the storage conditions.

The three above described solutions were subsequently shipped to a group of ca. 30 laboratories for an interlaboratory study which allowed to explain variations in standard deviations for ion chromatography due to e.g. the application of different columns, different eluents, the use of chemical or electronic suppression (conductivity detection) etc. This intercomparison actually allowed to constitute a group of experts and to prepare them for the certification campaign. In addition, this study enabled to confirm the suitability of the procedure used for the preparation of candidate

CRMs of verified homogeneity and stability. The results of this exercise are described in details elsewhere [5].

8.2.3. Production of the candidate reference materials

Two 150 L PVC containers (one for each solution) to be used for the preparation and homogenisation of the candidate reference materials were cleaned with a detergent, rinsed with distilled water and further rinsed with ultrapure water. For each of the reference materials, the ampoules were cleaned in a similar way. The ampoules were (air) dried for two days and conditioned for at least 24 h with the solution which they would contain. The preliminary investigations had shown that this procedure was adequate to bring the walls in adsorption equilibrium with the solution.

The sample preparation followed the procedure used in the preliminary investigations. The two reference materials were prepared from ultrapure water to which freshly prepared solutions of the different substances described in Table 8.5 were added (amounts in g added to approx. 150 L ultrapure water). All reagents were of pro analysis quality. The final pH of the solutions was ca. 6.8. The homogenisation of the solutions was achieved by maintaining a constant agitation (with a mechanical shaker) during the addition of the solutions. The (conditioned) ampoules were filled with the CRM solutions and immediately heat-sealed. The ampoules were then stored at ambient temperature in the dark. Precautions were taken to avoid contamination during the ampouling procedure.

The between-ampoule homogeneity was verified by determining nitrate by photometric detection [5]. The method was based on the reduction of nitrate to nitrite and the formation of diazo-compound which is coupled to sulphonylamide (SPA) and N-(naphthylethylene diamine, NED) to yield an azo derivative with a maximum optical absorption at 540 nm. After mixing with the carrier ($\text{NH}_4\text{Cl}/\text{Na}_2\text{B}_4\text{O}_7/\text{EDTA}$), the sample reaches the redox Cd column (copperized with $\text{EDTA}/\text{CuSO}_4$) where nitrate is reduced to nitrite. Then, the reduced analyte solution is sequentially mixed with the SPA and NED solutions to yield the azo compound, which is monitored at 540 nm as it passes through the flow cell of the photometric detector. The measured signal is the height of the FIA peak. The study enabled to conclude that both batches of CRMs were homogeneous, within the uncertainty of the method.

TABLE 8.5

AMOUNTS OF SUBSTANCES (GRAMS) ADDED TO 150 LITRES OF WATER FOR THE PREPARATION OF THE TWO CANDIDATE CRMS 479 AND 480

KNO_3	3.670 and 12.475
Na_2CO_3	79.500
$\text{CaCl}_2, 4\text{H}_2\text{O}$	13.755
$\text{MgSO}_4, 7\text{H}_2\text{O}$	18.450
Potassium phthalate	0.750
Lauryl sulphate	0.300
Phenylmercury acetate	1.500

The stability tests were performed by analysing randomly selected samples after 1, 3, 6 and 12 months of storage at +4, +20 and +40°C. No instability could be demonstrated and the materials were considered suitable for certification [4].

8.2.4. Certification

Pretreatment procedures included reduction on Cd-column, reduction by hydrazine addition with copper catalyst, or addition of NH_4Cl buffer. The final determination was performed by e.g. SPEC of diazo-compound at 540 nm or IC (conductivity). A detailed description of the techniques is given in the certification report [4].

Variations of 5% in the efficiency of the Cd-column are not uncommon due to inhomogeneity of the column; higher variations were, however, found unacceptable for certification. Doubts were expressed on the use of bicarbonate-carbonate buffer in comparison with borate buffer, the latter giving more reliable results.

Discussions arose on the apparent discrepancy within the different SPEC results for the CRM 480. No explanation could, however, be found. Since no systematic error was suspected the data were accepted for certification.

A further assessment of the sets of data was carried out using a Youden's plot [4,5] which showed clearly the prevalence of systematic errors. However, As discussed below, these systematic errors were not statistically significant and no differences could be observed, on statistical grounds, between the different techniques used.

With respect to the coefficient of variation (CV%) of the mean of laboratory means, the values obtained for both CRMs were in good agreement with precision usually obtained in a single laboratory for this range of concentrations [5].

The certified values of nitrate were respectively $(13.3 \pm 0.3) \text{ mg kg}^{-1}$ for CRM 479 and $(54.9 \pm 0.8) \text{ mg kg}^{-1}$ for CRM 480 (16 sets of results) [4,5].

The comparison of SPEC and IC showed no particular bias that could be attributed to one of the two techniques since the CVs within one method were systematically larger than those of different techniques (Table 8.6)

TABLE 8.6

RESULTS OF THE EVALUATION OF CONSISTENCY OF THE METHODS FOR NITRATE IN CRMS 479 AND 480

CRM	Techn. of final determination	CV (%) between means of lab. with the same technique	Nr of sets of results	CV (%) between means of techniques
CRM 479	SPEC	3.12	8	1.23
	IC	3.67	8	
CRM 480	SPEC	3.25	8	0.61
	IC	1.94	8	

8.2.5. Participating laboratories

The preparation, homogeneity and stability studies were carried out by the University of Córdoba, Department of Analytical Chemistry (Córdoba, Spain). The following laboratories participated in the certification campaign: Anglian Water, Colchester (United Kingdom); Centre d'Estudis Avançats de Blanes, Girona (Spain); C.N.R., Istituto Italiano de Idrobiologia, Pallanza (Italy); C.N.R., Istituto di Ricerca sulle Acque, Brugherio (Italy); CNRS., Service Central d'Analyse, Vernaison (France); Compagnie Générales des Eaux, Maisons-Lafitte (France); Dansk Teknologisk Institut, Århus (Denmark); Energieonderzoek Centrum Nederland, Petten (the Netherlands); Empresa Portuguesa das Aguas Livres, Laboratorios Centrais, Lisboa (Portugal); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Instituto Hidrográfico, Lisboa (Portugal); K.I.W.A., Nieuwegein (the Netherlands); Ministère des Affaires Economiques, Brussels (Belgium); Presidio Multizonale di Prevenzione, Venezia (Italy); University of Plymouth, Dept. of Environmental Sciences, Plymouth (United Kingdom).

8.3. MAJOR ELEMENTS IN RAINWATER

8.3.1. Introduction

From about 1975 rainwater has received increasing attention resulting from the concern on acid deposition. In Europe monitoring of rainwater is carried out in many Member States individually as well as on European scale, e.g. the ECE/EMEP-network (Economic Commission for Europe / Environmental Monitoring and Evaluation Programme). Components such as Al, Ca, Cl, Fe, Mg, Mn, P, K, Na and S are regularly determined in laboratories in the Member States in order to assess the impact of acid deposition on the environment. It has however been demonstrated that a large number of errors may occur in the analyses of rainwater [6]. The need for improvement of the quality of these determinations, has led the BCR to produce two certified reference materials (CRMs) of rainwater, one with a low (CRM 408) and one with a high mineral content (CRM 409) [3,7].

8.3.2. Production of the reference materials

Five 60 L polypropylene containers to be used for the preparation and homogenisation were rinsed with ultra pure water, interconnected, provided with a drain tap at the bottom and filled with hydrogen peroxide (30 g kg^{-1}) to disinfect. After a contact period of 6 h the hydrogen peroxide solution was circulated in the tanks by pumping continuously for 16 h. The tanks were emptied and rinsed with ultra pure water. Samples of the water and from the walls of the tanks were bacteriologically examined (diluted broth agar medium, 10 days at 25°C). Samples of the rinsing water had typical microbial number concentrations (CFP) [3] of less than 1 cm^{-3} . For the examination of the walls

a swab was taken with cotton wool; typical areic numbers (CFP) were less than 0.01 cm^{-2} . No contamination by algae could be detected by microscopic examination.

The drain tap of each tank was also cleaned with hydrogen peroxide (30 g kg^{-1}), leaving the tap filled for at least 2 h. The PTFE tip which was mounted to the drain tap for filling the ampoules was cleaned in the same way. Just before filling the PTFE tip was heat-sterilised. A test was carried out to estimate the losses by evaporation during the preparation of the reference material. The test consisted of filling three ampoules with ultra pure water. After weighing, the ampoules were left at room temperature for a period of 7 days. The relative loss by evaporation was less than 0.02% /day for both types of ampoules (diameter of 40 and 30 mm for respectively CRMs 408 and 409).

To establish possible contamination from the quartz ampoules, the water that was in contact with the quartz for 7 days was analysed. The analyses resulted in concentrations of the determinands below the limits of detection of the methods applied. For each of the reference materials, 1200 ampoules were conditioned for at least 24 h with the solution which they would contain. During conditioning, the ampoules were loosely closed with a synthetic foil. Preliminary experiments had shown that one treatment to bring the walls in adsorption equilibrium with the solution was sufficient.

The two reference materials were prepared from ultra pure water ($0.05 \mu\text{S m}^{-1}$), to which freshly prepared solutions of ammonium sulphate, sodium nitrate, magnesium nitrate hexa hydrate, sodium chloride, calcium chloride (all reagents: pro analysis quality), hydrochloric acid and ultra pure nitric acid were added.

To prevent air from the laboratory to enter into the containers a slight flow of pure nitrogen (produced from liquid N_2 passed over a column of active coal) was passed over the liquid surface in each container. Homogenisation of the solution was performed in the polypropylene containers, which were connected in series with PTFE tubing and covered with a close fitting polyethylene lid. A centrifugal pump connected to the containers with PTFE piping ensured constant circulation of the solution. The pump had no metal parts in contact with the solution.

After homogenisation for 24 h, both CRM solutions were examined for bacterial contamination. For both CRMs a value far below 100 CFP mL^{-1} (Colony Forming Particles per mL) was found, which generally is considered to be adequate.

The ampoules were filled with the CRM solution and left in contact with the solution for at least 24 h. After the conditioning each ampoule was emptied, filled with the solution and immediately heat-sealed. The samples were sterilised by gamma-irradiation with a ^{60}Co source, dose 25 kGy . After irradiation the ampoules were stored at ambient temperature in the dark. Some differences were observed in the contents before and after irradiation, particularly in the CRM 408 for hydronium, ammonium and nitrate. It was suspected that nitrate could have been formed from ammonium upon irradiation. Nitrite was also detected immediately after irradiation but was probably rapidly oxidised.

Analyses for verifying the between-ampoule homogeneity were performed after the gamma treatment to account for possible irradiation induced chemical changes. The analytical methods used were potentiometry (hydronium), UV-visible light spectrometry (ammonium), FAAS (calcium, potassium, magnesium, sodium) and IC (chloride, nitrate, sulphate). No significant between ampoule variability could be observed which enabled to conclude that both batches were homogeneous, within the uncertainty of the methods.

TABLE 8.7

SUMMARY OF THE TECHNIQUES USED IN THE CERTIFICATION OF CRMS 408 AND 409

Element	Techniques
Ca	DCP-AES, FAAS, IC, ICP-AES, IDMS, MS
Cl	COUL, GRAN, INAA, IC, SPEC, SPIT, TITR
H ₃ O ⁺	GRAN, POT, TITR
K	DCP-AES, FAAS, FAES, ICP-AES
Mg	DCP-AES, FAAS, IC, ICP-AES, ICPMS, IDMS, ZETAAS
Na	DCP-AES, FAAS, FAES, IC, ICP-AES, ICPMS, INAA
NH ₄	COND, IC, MS, SPEC
SO ₄	IC, ICP-AES, IDMS, SPEC

The stability tests were performed by analysing 6 randomly selected samples after 6, 15, 26, 52, 69 and 84 weeks of storage at ambient temperature in the dark. The same analytical procedures as for the study of homogeneity were used. No instability could be demonstrated and the materials were considered suitable for certification [7].

8.3.3. Certification

Various analytical techniques were used in the certification, involving different types of pretreatment. The methods of final determination are summarised in Table 8.7.

Certification of ammonium in CRM 408 was impossible due to a high spread in the results and the suspicion of a method bias. In general, the results obtained by ion chromatography were higher than those obtained by spectrometric methods.

The relatively high scatter of the chloride results obtained by titrimetry was due to problems related to the endpoint detection. There was no reason to suspect that the results deviate systematically and hence the results were accepted.

In the case of nitrate, one laboratory observed that the activity of the Cu-catalyst on the Cd-reductor was rapidly inhibited (change of colour to black). They attributed this to the possible presence of oxidising compounds remaining from the H₂O₂ added in the preparation or resulting from the irradiation process. The results of this laboratory were withdrawn. Owing to the redox properties of the sample (subject to further research) a copperised Cd-column is not recommended. A non-copperised Cd-reduction can be used.

The coupling of a flow injection system to FAAS for potassium resulted in a much smaller standard deviation than if a continuous flow FAAS system was applied. In CRM 408 and even in CRM 409 the latter system worked closer to its limit of determination.

The certified values are given in Table 8.8.

The comparison of methods for some elements showed that no particular bias could be attributed to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 8.9)

TABLE 8.8A

CERTIFIED VALUES FOR CRM 408

Element	Certified value	Uncertainty	Unit	p*
Calcium	7.68	0.11	$\mu\text{mol kg}^{-1}$	18
Chloride	67.3	0.7	$\mu\text{mol kg}^{-1}$	20
Hydronium	16.6	2.1	$\mu\text{mol kg}^{-1}$	6
Magnesium	6.14	0.14	$\mu\text{mol kg}^{-1}$	19
Sodium	42.0	0.6	$\mu\text{mol kg}^{-1}$	17
Nitrate	20.1	0.4	$\mu\text{mol kg}^{-1}$	18
Sulphate	10.5	0.3	$\mu\text{mol kg}^{-1}$	18

TABLE 8.8B

CERTIFIED VALUES FOR CRM 409

Element	Certified value	Uncertainty	Unit	p*
Calcium	15.5	0.3	$\mu\text{mol kg}^{-1}$	18
Chloride	113	2	$\mu\text{mol kg}^{-1}$	20
Hydronium	48.0	2.1	$\mu\text{mol kg}^{-1}$	5
Potassium	4.25	0.21	$\mu\text{mol kg}^{-1}$	12
Magnesium	12.3	0.2	$\mu\text{mol kg}^{-1}$	18
Sodium	82.9	1.2	$\mu\text{mol kg}^{-1}$	18
Ammonium	106	2	$\mu\text{mol kg}^{-1}$	14
Nitrate	78.1	1.0	$\mu\text{mol kg}^{-1}$	19
Sulphate	53.2	0.7	$\mu\text{mol kg}^{-1}$	20

* number of data sets

8.3.4. Participating laboratories

The preparation, homogeneity and stability studies of the reference materials were carried out by KIWA in Nieuwegein (The Netherlands). Certification analyses were carried out by the following laboratories: KIWA, Nieuwegein (The Netherlands); CNR, Istituto Italiano di Idrobiologia, Pallanza (Italy); CNRS, Service Central d'Analyse, Vernaison (France); Universidad Complutense, Departamento de Química Analítica, Madrid (Spain); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Empresa Portuguesa das Águas Livres, Lisbon (Portugal); Instituto Hidrográfico, Lisbon (Portugal); Institute of Freshwater Ecology, Dorset (United Kingdom); Institute for Occupational Health, Bilthoven (The Netherlands); Institute of Terrestrial Ecology, Edinburgh (United Kingdom); Institute of Terrestrial Ecology, Merlewood (United Kingdom); Laboratory of Industrial Hygiene, Pavia (Italy); Laboratory Pascher of Microanalysis, Remagen-Bandorf (Germany); Ministère des Affaires Economiques, Brussels (Belgium); Serviços Nacionais dos Parques, Lisbon (Portugal); Portsmouth

TABLE 8.9A

RESULTS OF THE EVALUATION OF CONSISTENCY OF THE METHODS USED FOR CRM 408

Compound	Techn. of final determination	CV (%) between means of lab. with the same technique	Nr of sets of results	CV (%) between means of diff. techniques
Calcium	FAAS	4.16	7	0.79
	ICP	1.77	6	
Chloride	IC	1.87	11	0.46
	SPIT	2.12	3	
Magnesium	FAAS	3.26	7	0.51
	ICP	2.85	5	
	ICPMS	5.99	3	
Sodium	FAAS	3.42	6	0.25
	ICP	1.43	4	
nitrate	IC	2.71	10	1.61
	SPEC	3.61	8	
sulphate	IC	4.07	12	1.70
	ICP	5.69	3	

Polytechnic, Portsmouth (United Kingdom); Laboratoire Régional d'Hydrologie, Illkirch (France); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Risø National Laboratory, Roskilde (Denmark); Rothamsted Experimental Station, Harpenden, Herts (United Kingdom); Universidad de Córdoba, Departamento de Química Analítica, Córdoba (Spain); Universiteit Gent, INW, Ghent (Belgium); Warren Spring Laboratory, Stevenage (United Kingdom); Swedish Environmental Protection Agency, Uppsala (Sweden).

8.4. TRIMETHYLLEAD IN RAINWATER

8.4.1. Introduction

Environmental contamination by lead is widespread, the major anthropogenic source of this element being the combustion of leaded gasoline. Although their use has been discontinued in some countries, the use of tetraalkyllead compounds as antiknock agents remains the largest application of organolead compounds [8]. It is recognised that, owing to its ubiquity and concern over the toxicity of organolead compounds in the environment, the monitoring of lead species will need to be continued over the next decade. Vehicular emissions of tetraalkyllead are subject to atmospheric breakdown to trialkyl- and dialkyllead and all three forms are scavenged from the atmosphere by rainfall [9]. Therefore, trimethyl- and triethyllead are found in road drainage and surface

TABLE 8.9B

RESULTS OF THE EVALUATION OF CONSISTENCY OF THE METHODS USED FOR CRM 409

Compound	Techn. of final determination	CV (%) between means of lab. with the same technique	Nr of sets of results	CV (%) between means of diff. techniques
Calcium	FAAS	2.98	7	1.42
	ICP	3.11	6	
Chloride	IC	2.88	11	0.83
	SPIT	1.29	3	
Magnesium	FAAS	1.61	6	0.40
	ICP	1.59	5	
	ICPMS	6.03	3	
Sodium	FAAS	0.53	5	0.10
	ICP	2.20	5	
Ammonium	IC	3.63	4	0.42
	SPSA	1.54	6	
Nitrate	IC	2.53	10	0.32
	SPEC	1.84	8	
Sulphate	IC	3.28	12	0.58
	ICP	3.23	3	

water. As a consequence, a number of laboratories are performing analyses of e.g. rainwater and urban dust to monitor the levels of trialkyllead compounds in the environment. In order to respond to the need for CRMs for QC purpose, the BCR has recently produced an urban dust reference material certified for its trimethyllead content, CRM 605 (see chapter 11); this section describes the attempt to certify a reference material of artificial rainwater (RM 604) for its trimethyllead content.

8.4.2. Feasibility study

One of the most critical points in organometallic chemistry analysis is the availability of calibrants of suitable purity and verified stoichiometry. This aspect was recognised at an early stage of the project and the purity of alkyllead compounds used in the feasibility study was carefully verified [10]. Additional experiments were performed on calibrants in the frame of the first interlaboratory exercise as described below.

Trimethyl- (TriML) and triethyllead (TriEL) compounds were obtained from Alfa products (Johnson Matthey) and their purity was verified as follows: carbon, hydrogen and chloride relative masses in the TriML and TriEL calibrants were determined by elemental microanalysis; the chloride concentration was determined by ion chromatography. Total lead was determined in the calibrants by electrothermal atomic absorption (ETAAS) using two different acid digestion procedures (concentrated nitric

acid and mixture of nitric acid/hydrogen peroxide). Calibrant solutions of TriML and TriEL at the 25 mg L^{-1} level were prepared in distilled deionized water (DDW) and analysed; a $200 \text{ }\mu\text{L}$ aliquot of each of these solutions was added to a solution containing NaCl (2 g), 0.5 mol L^{-1} NaDDTC (2 mL) and 0.1 mol L^{-1} EDTA (1 mL) in 30 mL DDW, and the mixtures were shaken manually in a separating funnel. Hexane (5 mL) was added to the funnel and the aqueous phase was removed shaking for 4 minutes. The extracted alkyllead compounds were then re-extracted into dilute nitric acid and hydrogen peroxide, and determined by ETAAS.

An aqueous solution containing 500 ng L^{-1} of TriML and TriEL was prepared and extracted as above (except for the addition of nitric acid and hydrogen peroxide). The hexane extract was transferred to a 25 mL conical flask and 0.5 mL propyl magnesium chloride (Grignard) reagent was added, followed by gentle shaking for 8 min. The extract was then washed with $0.5 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ (5 mL) to destroy any excess Grignard reagent present. The organic phase was separated and dried with a minimum of anhydrous Na_2SO_4 and transferred to a 4 mL vial. TriML and TriEL were determined by gas chromatography (GC-AAS). Students t-tests were used to compare the experimental results obtained with the predicted values.

The results showed that the content of alkyllead in the calibrants was slightly less than 100% and that extraction/analytical losses were the likely causes for this, rather than inorganic lead (since this was not significantly different from the expected concentration). In addition, if there was contamination by inorganic PbCl_2 in the standards, the concentration of chloride obtained by microanalysis and ion chromatography, would have been higher than expected. In the two chloride analyses, it was found that the chloride concentration was slightly lower than expected, probably due in part to the tetraalkyllead detected in the calibrants (around 2% of the total lead content). In the light of these results, it was thought unlikely that significant amounts of either inorganic lead or other ionic alkyllead compounds were present. It was concluded, therefore, that the two trialkyllead calibrants were not less than 98% pure.

8.4.3. Interlaboratory studies

For the first intercomparison exercise, participants were asked to dilute the solution 1000 fold, i.e. to determine levels of TriML of ca. $40 \text{ }\mu\text{g L}^{-1}$. Some laboratories also analysed the solutions after a 10,000-fold dilution. The participating laboratories in the second interlaboratory study received two sets of solutions containing ca. 50 and $5 \text{ }\mu\text{g L}^{-1}$ of TriML respectively. They were requested to perform five replicate analyses of, respectively:

- 10-fold dilution of the $50 \text{ }\mu\text{g L}^{-1}$ concentrated solution (solution A);
- 10-fold dilution of the $5 \text{ }\mu\text{g L}^{-1}$ concentrated solution (solution B);
- 100-fold dilution of the $5 \text{ }\mu\text{g L}^{-1}$ concentrated solution (solution C);
- 1000-fold dilution of the $5 \text{ }\mu\text{g L}^{-1}$ concentrated solution (solution D).

Preliminary investigations have shown that ethyllead compounds are more sensitive towards degradation than the corresponding methylated compounds [10]; trimethyllead was found to be stable in solutions kept at ambient temperature in the dark. Consequently, a batch of solutions containing 40 mg L^{-1} of trimethyllead chloride (as Pb) and 100 mg

L⁻¹ of lead nitrate (as Pb) added as interferent was prepared and the stability was verified over a period of six months.

Two sets of simulated rainwater solutions were prepared. Aliquots of solution (100 mL) were transferred into eighteen 100 mL Nalgene bottles for each of the samples. The bottles were wrapped with aluminium foil and then sealed in plastic bags.

In most cases, the pretreatment techniques used were based on complexation, GC separation and employed various different detection techniques (e.g. MIP-AES, AAS, ICP-MS). No specific comments were made during the meeting to discuss the first interlaboratory study, except that the participants considered it impossible to correct the results for impurities in the calibrant matrix.

The second interlaboratory study generated detailed discussions: one ETAAS technique employed did not include a separation step but the participant stated that EDTA extraction would only extract organic lead compounds; this technique was considered to be suitable for the analysis of a simple solution containing only one lead compound but would not be suitable for mixtures of lead species, e.g. the technique would not allow the separation of TriML and TriEL in a natural rainwater sample. In cases where different organolead compounds are to be determined in natural samples or solutions containing different lead compounds, ETAAS should be coupled to a separation technique, e.g. GC or HPLC.

A systematic difference was observed by one laboratory between two different sets of calibrants (calibrant solution made with a newer calibrant from the same producer). This highlighted the need to thoroughly verify the calibrant, i.e. not to rely on calibrants from one producer of which the quality could vary from one set to another. Most of the laboratories actually used their own calibrants, which were not verified for purity and stoichiometry. Only one laboratory used the calibrant previously verified and distributed in the first interlaboratory study [8]. It was stressed that calibration was an important issue and that more efforts should be put on the verification of calibrants in future exercises. It was agreed that the coordinator of the project would purchase calibrant from a chemical company and establish its purity; sets of verified primary calibrants would then be made available to participants in a further exercise to characterise their own calibrants.

The verification of extraction recoveries was also discussed. Most of the laboratories performed standard addition procedures and hence did not need to correct for recovery.

The Coefficient of Variation (CV) between laboratories was originally 20.9%. After technical scrutiny and rejection of suspect data, the CV decreased to ca. 4%, which was found to be an excellent agreement.

8.4.4. Production of the candidate CRM

The composition of the artificial rainwater matrix was chosen to reflect that of natural rainwater which falls over continental land masses [11]. A stock solution was prepared at 100 times the required concentration of the artificial rainwater by the addition of inorganic compounds to deionised water. One litre of stock solution was prepared by the addition of the following compounds (Analytical reagent grade) to 1000 mL of Milli-Q deionised water:

CaCl ₂	113 mg
MgCl ₂	95 mg
(NH ₄) ₂ SO ₄	396 mg
NaNO ₃	340 mg
NaCl	117 mg
KCl	37 mg
HCl	174 µL

The components were dissolved using an ultrasonic bath and the final solution was filtered through a 0.2 µm (47 mm diameter) cellulose acetate membrane in order to eliminate algal and bacterial particles. No problems were encountered with solubility of any of the components of the stock solution.

Trimethyllead chloride (purity 98%) was used for spiking the candidate CRM. A large batch of artificial rainwater (100 L) was prepared in a rigid high-polythene bin (0.14 m³) with a lid. The solution was protected from light by wrapping the bin in aluminium foil. Trimethyllead chloride was added to give a concentration of 500 ng L⁻¹ (as lead). The solution was thoroughly stirred for an extended period of time to ensure proper mixing by means of a glass rod which was passed through a hole in the lid of the container. The solution was dispensed into Nalgene bottles (125 mL) by syphoning it through a Teflon tube (6.4 mm diameter) which was passed through the hole in the container lid. The Nalgene bottles (800 units) were rinsed out with two small aliquots of the solution and 100 mL of the artificial rainwater matrix was dispensed to each bottle. The bottles were capped, wrapped in aluminium foil, sealed in polythene bags and then stored in a cold room at +4°C.

The ionic strengths of the components present in the stock solution and in the artificial rainwater are presented in Table 8.10.

To verify the between-bottle homogeneity, the artificial rainwater samples (100 mL) were diluted with 900 mL of Milli-Q deionised water prior to analysis. NaCl (20 g), 5 mL of 0.25 mol L⁻¹ NaDDTC and 5 mL hexane were added to the diluted sample and the solution was shaken mechanically for 30 min. The organic fraction was transferred

TABLE 8.10

CONCENTRATION OF ARTIFICIAL RAINWATER COMPONENTS

Component	Concentration in stock solution (mmol L ⁻¹)	Concentration in final solution (µmol L ⁻¹)
NH ₄ ⁺	6	60
K ⁺	0.5	5
Ca ²⁺	1.2	12
Mg ²⁺	1	10
Na ⁺	6	60
Cl ⁻	9	90
SO ₄ ²⁻	3	30
NO ₃	4	40
(CH ₃) ₃ Pb ⁺	500 ng kg ⁻¹	50 ng kg ⁻¹

to a 25 mL conical flask, 0.5 mL propylmagnesium chloride was added and the flask was gently shaken for 8 min. The extract was washed with 5 mL of 0.5 mol L⁻¹ H₂SO₄ to destroy any excess Grignard reagent present. The organic phase was dried over anhydrous Na₂SO₄ (ca. 100 mg) and then transferred to a 4 mL vial. A 50 µL extract was injected into a GC and trimethyllead was determined by AAS. The extraction efficiency for trimethyllead was determined by spiking the sample with 100 ng L⁻¹ of the compound and performing the analysis as described above. The mean recovery for 4 replicates was 90.2% with a relative standard deviation of 2.9%. No significant differences were observed between the between-bottle and method variances. On the basis of these results, no inhomogeneities of the material were suspected.

The stability of the trimethyllead content was tested at 4°C over a period of 12 months of storage in the dark and trimethyllead was determined at regular intervals during the storage period. Analyses were repeated after 37 months to verify the long-term stability. In addition, a short-term stability study was carried out at +37°C to simulate worst-case transport conditions. While the trimethyllead content did not show any instability over a period of 12 months, the results indicated that the difference between the initial concentration of trimethyllead measured on the day of the rainwater preparation and the final concentration after 37 months storage was significant at the 95% confidence level. On average more than 10% of trimethyllead in rainwater has decomposed at 4°C after three years storage. In addition, the data of the short-term stability study showed that trimethyllead had decomposed significantly after 15 days storage at +37°C.

8.4.5. Certification

Techniques used by the participating laboratories were in most cases composed of different analytical steps (extraction, derivatisation, separation and detection), illustrating the high diversity of hyphenated techniques developed for Pb-speciation analysis. The analytical techniques applied in this exercise are summarised in Table 8.11.

A trimethyllead calibrant was prepared by the University of Plymouth for the purpose of the certification campaign in order to enable participating laboratories to verify their own calibrants. a portion of 39 g of tetramethyllead and toluene (80% w/w) was placed in a round bottom flask and hexane (250 mL) was added. Dried hydrogen chloride gas was bubbled through the mixture for 10 min at a flow rate of 150 mL min⁻¹. A heavy white precipitate was formed and was removed by filtration; it was first washed with hexane (300 mL) and finally rinsed with pentane before being dried under reduced pressure. The original reaction mixture was discarded, the apparatus was cleaned with hexane and a fresh preparation was undertaken in order to produce sufficient trimethyllead chloride for the purpose of the project. The purity of the product was assessed using NMR spectroscopy and was found to be greater than 99% [9].

The technical discussion focused firstly on the calibration methods used by the participants. All laboratories used the trimethyllead calibrant provided by the University of Plymouth, either for calibration or verification of their own calibrants. It was noted that some deterioration had been observed in a commercial calibrant over a two-year period. No significant difference was observed by the laboratories between external

TABLE 8.11

Techniques used

EDTA complexation, hydride generation (with NaBH_4) and ZETAAS detection (this method is only appropriate for differentiation between organic and inorganic lead)

Hexane extraction, DDTC complexation, Grignard derivatization (propylation), CGC separation and QFAAS detection

NaOH extraction, derivatization with NaBEt_4 , CGC separation and QFAAS detection

Hexane extraction, Grignard derivatization (propylation), packed GC (U-tube) and QFAAS detection

EDTA complexation, derivatization with NaBEt_4 followed by hexane extraction, CGC separation and MIP-AES detection

EDTA buffer, Grignard derivatization (propylation), CGC separation and ICPMS detection

EDTA and DDTC buffer, Grignard derivatization (butylation), CGC separation and ICPMS detection

EDTA complexation, derivatization with NaBEt_4 , CGC separation and ID-ICPMS detection

NaDDTC complexation, hexane extraction, Grignard derivatization (pentylation), CGC separation and MS detection

NaDDTC complexation, pentane extraction, Grignard derivatization (butylation), CGC separation and MS detection

Removal of inorganic Pb and DPASV determination

calibration and standard addition. Some laboratories used tetraethyllead or tributyllead as internal standard.

With the exception of three laboratories which used, respectively, a hydride generation method, DPASV and HPLC, all the participants had used a gas chromatographic separation following a Grignard derivatisation of the analyte. Some differences were observed in terms of precision by two laboratories using the same separation and detection but different Grignard reagents; in one case, some losses were suspected during pentylation by Grignard reagent, whereas, in another case, a butylation method resulted in a cleaner reaction, thereby accounting for the discrepancy in precision. In the subsequent discussion, it was agreed that the conditions of the Grignard reaction in terms of temperature, concentration and length of the alkyl chain were key factors which require careful control. The longer the alkyl chain of the Grignard reagent, the greater the risk of degradation product formation and peak broadening in the chromatographic stage.

While the results obtained by the participating laboratories were in good agreement and illustrated the high quality of the measurements performed [12], the doubts expressed on the stability of the reference material did not encourage the BCR programme to recommend this material for certification. It is thought that such material could be certified, providing that storage at low temperature ($+4^\circ\text{C}$ or below) in the dark would

be constantly maintained; in the case of the RM 604, the good results obtained over 12 months at +20°C were obviously not sufficient since the material was shown not to be stable after 37 months.

8.4.6. Participating laboratories

The sample preparation, homogeneity and stability studies were performed by the University of Birmingham, School of Chemistry. The following laboratories participated in the certification: GALAB, Geesthacht (Germany); Institut für Chemo- und Biosensorik, Münster (Germany); Technische Universität Wien (Austria); Universiteit Antwerp, Dept. Scheikunde, Wilrijk (Belgium); University of Birmingham, School of Biological Sciences, Birmingham (United Kingdom); Universität Mainz, Abteilung Anorganische Chemie, Mainz (Germany); University of Plymouth, Dept. Environmental Sciences, Plymouth (United Kingdom); University of Umeå, Dept. of Chemistry, Umeå (Sweden); Universidad de Zaragoza, Centro Politécnico Superior de Ingenieros, Zaragoza (Spain); Vrije Universiteit Amsterdam, Inst. Milieuvraagstukken, Amsterdam (The Netherlands).

8.5. MAJOR ELEMENTS IN GROUNDWATER

8.5.1. Introduction

Groundwater is currently monitored by EU laboratories to control the level of contamination by major or trace elements, in particular in support of EC Directives 80/68/CEE, 80/778/CEE and 91/676/CEE which prescribe the determination of a variety of elements and compounds. The need to control the quality of measurements calls for the availability of CRMs of typical groundwater samples. Considering the difficulty of stabilising natural groundwater samples with respect e.g. to nutrients, it was decided to produce artificial reference materials representative of groundwater matrices. The parameters selected were based on a literature research [13] which enabled the definition of a mean composition for two types of materials with, respectively, low and high carbonate contents (CRM 616 and 617). This section describes the feasibility study on preparation and the certification of the reference materials for a range of major elements.

8.5.2. Feasibility study on sample preparation

The optimal conditions for the preparation of groundwater candidate CRMs were tested in a feasibility study of which the results are published elsewhere [14]; two materials were selected, representing typical carbonate and sandstone media. Two batches were prepared from ultrapure water by adding pro-analysis grade chemicals and their homogeneity was verified to evaluate possible effects of the preparation procedure on the sample composition [14]. The stability was also checked at +4°C and +20°C over a period of three months.

The precipitation of CaCO_3 and Fe-oxides was considered to be a possible source

of inhomogeneity and instability. The pH of the solution is mainly governed by the dioxide/bicarbonate/ carbonate equilibrium and it was considered to be critical for the stability. The pH was therefore lowered by adding citric acid in order to achieve a pH not higher than 7. The metabolic activity of micro-organisms may also affect some sample constituents, changing their oxidation state. It can be responsible for changes in e.g. nitrate, nitrite, ammonia and phosphate contents. In order to inhibit microbial activity, sample sterilisation by gamma-irradiation and by autoclave at 120°C was tested. With respect to irradiation, it was observed that radiolysis of water affected the contents of the nitrogen cycle components which was reflected by an increase in ammonium content, a decrease in nitrate content, and the formation of nitrite. Autoclave sterilisation did not lead to such changes, which justified the choice of this procedure for the preparation of candidate CRMs [14].

The addition of UV-absorbing organic matter was also discussed to match the presence of humic acids. An attempt to add humic acids to the samples failed, owing to a decomposition of these acids during autoclave sterilisation. It was hence decided to add lauryl sulphate as a compromise for matching the presence of these acids, as was successfully performed for the certification of nitrate in artificial freshwater [5]. The short-term stability study demonstrated that the samples remained stable under the conditions tested.

8.5.3. Production of the reference materials

The equipment used for the preparation and homogenisation of each candidate reference material consisted in a closed system designed to avoid microbiological and dust contamination; it was composed of a high density polyethylene container (150 L) and a magnetic drive pump which had no metal parts in contact with the solution. All rings and piping devices were made of polypropylene [14]. The container and connections were rinsed with ultrapure water and filled close to the brim with nitric acid solution (5% v/v). After a contact period of 12 h the nitric acid solution was circulated by pumping continuously for 1 h. The tank was rinsed with ultrapure water and refilled with hydrogen peroxide (3% v/v) for disinfection; the hydrogen peroxide solution was circulated in the tank for 2 h after a contact period of 6 h. The tank was then emptied and rinsed with ultrapure water. Samples of the rinsing water were collected in the container and the connections were bacteriologically examined (heterotrophic bacteria tested with the pour plate method); all the samples were found to be free from viable micro-organisms (tested after 24 h at 37°C and 3, 7 and 14 days at 22°C).

Borosilicate glass ampoules of 100 mL were used for the preparation of the reference solutions; they were washed with hot water, rinsed with ultrapure water, soaked during 24 h with 5% nitric acid solution and rinsed again with ultrapure water. The ampoules were then stoppered and sterilised at 180°C during 45 min. Blank tests performed in water in contact during 7 days with the ampoules did not reveal any chemical or microbiological contamination. The ampoules were not conditioned with the solutions they would contain in order to avoid bacterial contamination [14].

Two batches of artificial groundwater samples, corresponding to typical carbonate (CRM 616) and sandstone (CRM 617) media, were prepared from ultrapure water to

which freshly prepared solutions of ammonium chloride (both CRMs), calcium chloride (both CRMs), calcium hydroxide (CRM 616), calcium nitrate (CRM 616), magnesium chloride (both CRMs), magnesium nitrate (CRM 616), magnesium sulphate (both CRMs), manganese(II) sulphate (both CRMs), potassium nitrate (both CRMs), iron citrate (both CRMs), sodium hydrogen phosphate (both CRMs), sodium carbonate (CRM 616), sodium hydrogen carbonate (CRM 617), sodium sulphate (CRM 617) and lauryl sulphate were added. All reagents were of pro-analysis grade quality. The salt solutions were previously filtered through a 0.2 μm sterilised acetate cellulose membrane to reduce microbiological contamination risks. Additional details on this preparation are given in the certification report [15].

Homogenisation of the solutions was performed in the polyethylene container covered with a close fitting polyethylene lid. A magnetic drive pump connected to the container with polyethylene piping ensured a constant recirculation of the solution. To prevent air from the laboratory to enter the container a slight flow of pure nitrogen was passed over the liquid surface in the container. After homogenisation for 12 h, both CRMs were examined for possible bacterial contamination; for both materials a value far below 100 CFP mL^{-1} (Colony Forming Particles per mL) was found which was considered to be adequate for the purpose [15]. The ampoules were filled with the CRM solutions and immediately heat-sealed. The materials were sterilised by autoclaving at 120°C for 20 min and subsequently stored at ambient temperature (ca. 20°C) in the dark.

The analytical methods used to verify the between-ampoule homogeneity were potentiometry (pH), flame atomic absorption spectrometry (Ca, K, Mg and Na), colorimetry (NH_4 , NO_3 , PO_4 and SO_4) and electrothermal AAS (Fe and Mn). No significant differences were observed between the between-ampoules and method variances and the materials were hence considered to be homogeneous [15].

The stability tests were performed over 52 weeks storage at +4°C and +20°C. A short-term stability study was also carried out at +40°C to test worst-case transport conditions. No instability could be detected at any of the conditions tested [15].

8.5.4. Certification

Various analytical techniques were used in the certification, involving different types of pretreatment. The methods of final determination are summarised in Table 8.12.

Sources of errors that were detected were mainly due to calibration errors, high blanks explaining high results and uncontrolled interferences (e.g. in ICPMS).

Samples which were left opened and left during three days displayed precipitation phenomena which explained low results for chloride. It was hence recommended to analyse the materials immediately after opening the ampoules; this recommendation was supported by similar observations made for sulphate results.

For ammonium, some colorimetric techniques were too close from the detection limits, which explained the apparent spread of results. In addition, interferences in ion chromatography were observed with sodium. Considering the spread of results, the participants recommended that the ammonium values be given as indicative only in the certification report [15].

It was stressed that ICP-AES and ICPMS enabled total phosphorus to be determined

TABLE 8.12

SUMMARY OF THE TECHNIQUES OF FINAL DETERMINATION (CRMS 616/617)

Element	Techniques
Ca	FAAS, HR-ICPMS, ICP-AES, ICPMS
Cl	CZE, IC, POT, SPEC
Fe	ETAAS, FAAS, HR-ICPMS, IC, ICP-AES
K	FAAS, FAES, IC, ICP-AES
Mg	ETAAS, FAAS, HR-ICPMS, IC, ICP-AES, ICPMS
Mn	ETAAS, HR-ICPMS, ICP-AES, ICPMS
Na	FAAS, FAES, IC, ICP-AES, ICPMS
NO ₃	CZE, IC, SPEC
PO ₄	IC, SPEC
SO ₄	CZE, IC, ICP-AES, SPEC

whereas ion chromatography determined orthophosphate and colorimetry determined reactive phosphorus (both 'inorganic phosphate'). It was hence decided to certify the content of inorganic phosphate and to use the ICP-AES and ICPMS results as supporting results.

Ar-interferences with ³⁹K and ⁶⁴Fe were likely the cause for high standard deviations in ICPMS for potassium and iron, respectively.

In the case of CRM 617, losses of Fe were suspected to have occurred during the preparation, considering the significant difference of the mean of laboratory means ($(0.191 \pm 0.012) \text{ mg kg}^{-1}$ in comparison to 0.206 mg kg^{-1}). Precipitation of Fe(III) could be one cause or loss during filtration; participants rather suspected that adsorption occurred on the ampoule since two sets of results corresponding to solutions acidified in the ampoule yielded higher results. Since risks of release could occur in case of adsorption, participants recommended that the Fe value in CRM 617 be considered as indicative only.

The certified values are given in Table 8.13.

8.5.5. Participating laboratories

The preparation of the reference materials, as well as the homogeneity and stability studies, were performed by the Empresa Portuguesa das Águas Livres in Lisbon (Portugal). The analyses for certification were carried out by the following laboratories: Aristotle University, Lab. Analytical Chemistry, Thessaloniki (Greece); Compagnie Générale des Eaux, Anjou Recherche, Maisons-Lafitte (France); CSIC, Instituto J. Almera, Barcelona (Spain); Empresa Portuguesa das Águas Livres, Lisbon (Portugal); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Institut Scientifique de Service Public, Liège (Belgium); Istituto Italiano di Idrobiologia, CNR, Pallanza (Italy); Istituto di Ricerca sulle Acque, CNR, Brugherio (Italy); KIWA N.V., Nieuwegein (The Netherlands); Lyonnaise des Eaux, CIRSEE, Le Pecq (France); Service Central d'Analyse, CNRS, Vernaison (France); Swedish University of Agricultural

TABLE 8.13

CERTIFIED VALUES FOR CRMS 616 AND 617

p: sets of results (each of at least 5 replicates)

CRM 616	Certified value in mg kg ⁻¹	Uncertainty in mg kg ⁻¹	p
Ca	38.5	0.9	17
Cl	49.8	1.0	11
Fe	0.052	0.003	10
K	0.58	0.03	11
Mg	23.9	0.3	17
Mn	0.0197	0.0007	13
Na	61.5	0.7	17
NO ₃	50.4	0.9	10
PO ₄	3.36	0.13	11
SO ₄	57.3	1.1	10
CRM 617	Certified value in mg kg ⁻¹	Uncertainty in mg kg ⁻¹	p
Ca	14.6	0.4	18
Cl	26.4	0.4	11
K	9.93	0.26	14
Mg	7.32	0.15	18
Mn	0.050	0.002	16
Na	14.6	0.3	17
NO ₃	25.8	0.5	10
PO ₄	0.272	0.005	7
SO ₄	26.3	0.5	10

Sciences (Sweden); Technologiezentrum Wasser, Karlsruhe (Germany); Universidad Complutense, Depto. de Química Analítica, Madrid (Spain); Universiteit Gent, Instituut voor Nucleaire Wetenschappen, Ghent (Belgium); Water Quality Institute, Hørsholm (Denmark); Yorkshire Water, Alcontrol, Rotherham (United Kingdom).

8.6. TRACE ELEMENTS IN GROUNDWATER

8.6.1. Introduction

To complete the series of groundwater reference materials (see section 8.5), a separate project has been carried out, focusing on a range of trace elements and bromide in natural groundwater samples. In natural groundwaters, in addition to the relatively low concentrations of trace elements, the dissolved organic matter (DOM) is usually much

higher than in other types of water; this may result in a strong complexing behaviour due to this DOM which creates difficulties in the determination of trace element contents. Consequently, the present project started by an interlaboratory study to check the feasibility of material preparation and the analytical state of the art, and continued by a certification campaign of groundwater CRMs which were certified for their contents of Al, As, Cd, Cu and Pb (CRMs 609 and 610) and bromide (CRMs 611 and 612).

8.6.2. Interlaboratory study

A feasibility study was carried out to test the preparation of possible candidate groundwater reference materials, which was followed by an interlaboratory study involving 22 laboratories from 15 countries. Natural groundwater samples were selected on the basis of monitoring data obtained by the Danish Geological Survey within the last five years. Samples were collected at Danish waterworks. Samples for trace elements were filtered on-line during collection to avoid oxidation of the groundwater and were immediately acidified with nitric acid at pH 2; they were filled in polyethylene bottles previously cleaned with hot HCl and rinsed with Milli-Q water. Samples for bromide were filtered at 0.45 μm (Sartorius filter) and diluted with Milli-Q water; they were filled in pre-conditioned brown 25 mL ampoules and packed in boxes each containing 5 ampoules. The homogeneity and stability study were verified and found to be sufficient for the purpose of the interlaboratory study [16].

The techniques used in the interlaboratory study were basically the same as the ones applied for certification. The results obtained are summarised in Table 8.14. Some

TABLE 8.14

SUMMARY OF THE RESULTS OF THE INTERLABORATORY STUDY

Element	Groundwater Low trace element content				Groundwater High trace element content			
	nr. of sets	acc. sets	CV% (raw)	CV% (sel.)	nr. of sets	acc. sets	CV% (raw)	CV% (sel.)
Al	16	11	24.9	9.8	16	11	21.1	15.5
As	16	15	21.7	11.3	16	10	22.8	10.3
Cd	19	15	15.8	14.8	18	14	15.0	6.9
Cu	19	12	12.6	6.8	19	14	23.2	20.6
Ni	18	11	16.1	6.8	18	12	21.4	10.3
Pb	19	12	24.8	11.1	20	10	28.4	16.7
Bromide	9	5	29.5	20.4	9	7	13.4	12.3
Iodide	6	3	157.7	15.8	6	3	96.9	14.5

nr. of sets: total number of data sets

acc. sets: sets accepted after technical scrutiny

CV% (raw): coefficient of variation of the mean of lab. means for all data sets

CV% (sel.): coefficient of variation of the mean of lab. means of selected data sets

recommendations could be drawn from these: the risk of incomplete extraction due to the presence of complexing agents, e.g. for cadmium, could lead to low results which should hence be checked by repeated extractions. The problem raised by the presence of organic matter may be overcome in many cases by the use of UV irradiation or addition of acids. This possible pretreatment should be carefully investigated prior to analysing the sample. Low results found by DPASV may be related to a strong effect of organic compounds, which are not completely destroyed by UV irradiation (e.g. organic compounds originating from agricultural waste). A way to overcome this problem is to add H_2O_2 before UV irradiation.

Possible problems of interferences in ICP-MS were highlighted as well as the need to carry out a preconcentration step. Spectral interferences may occur for copper, leading to high results caused by spectral overlap from unidentified polyatomic species at mass 65. The existence of this species in natural samples was stressed in the certification of an estuarine water reference material by a mass spectrum obtained by high resolution ICPMS, although its exact composition could not be determined.

Table 8.14 illustrates the relatively high coefficient of variation (CV) obtained between laboratories for the raw data for some elements (e.g. Al, As, Pb). However, after the technical discussion and removal of obvious outlying values (most of them likely due to calibration errors), the CVs of the accepted sets of data were much more acceptable, ranging from ca. 7% to ca. 17% (with the exception of Cu). The participants agreed that this range of CVs was corresponding to the state of the art of natural groundwater analysis for trace elements and, consequently, recommended the organisation of a certification campaign. For bromide and iodide, it was concluded that, the state-of-the-art seemed to be sufficient for bromide to envisage certification; however, much would remain to be done before iodide may be proposed for certification [16].

8.6.3. Production of the reference materials

8.6.3.1. CRMs for trace elements

Four 680 L polyethylene containers (two for each solution) to be used for the sampling, preparation and homogenisation of the candidate CRMs were cleaned with detergent, rinsed with distilled water and soaked with diluted pro-analysis nitric acid (1:10). The containers were rinsed again with Milli-Q water and soaked with Milli-Q water for a period of at least one month prior to the use of the containers. Tubings used for filtration and homogenisation were also cleaned at the same time as the container cleaning procedure.

The filters used for filtration were of the types Sartorius depth filter (particle retention size 0.7 μm) and Sartorius PH membrane filter capsule (particle retention size 0.45 μm). Polyethylene bottles produced by Nalgene were used for each of the reference material. 2600 pieces of 500 mL bottles were cleaned, using the following procedure:

- (1) soak for 24 h in detergent solution, rinse with demineralised water;
- (2) soak for 1 week in diluted pro-analysis HNO_3 (1:4), rinse with demineralised water;
- (3) soak for 1 week in diluted HCl (1:4);
- (4) rinse with Milli-Q water;

- (5) conditioning with acidified water to $\text{pH} < 2$ (nitric acid, suprapure) until use of the bottles (minimum three months). Filled bottles were packed in plastic bags to prevent contamination during storage. Steps 2, 3 and 5 were controlled by regular sampling to detect possible contamination.

The sampling procedures was similar to the one described in section 8.6.2. A groundwater sample (680 L) for the preparation of CRM 609 was collected on 1st July 1996 at Sjaelsø Waterworks, Birkerød (Denmark). A groundwater sample (680 L) for the preparation of CRM 610 was collected on 3rd July 1996 at Kildeskovhallen, Gentofte (Denmark) from a borehole. The groundwater samples were filtered on-line through $0.7 \mu\text{m}$ Sartorius PE filter by use of a peristaltic pump system. Prior to sampling at least 30 L groundwater was pumped through the filtering system.

Both groundwater samples were acidified with suprapure nitric acid to $\text{pH} < 2$. The groundwater samples were left for more than one month after acidification. The samples were then filtered with a Sartobran PH membrane capsule filter (0.65 and $0.45 \mu\text{m}$ pore size) and pumped into another clean container. Freshly prepared solutions of trace elements were added to the samples to achieve concentration levels given in Table 8.15. The homogenisation of the samples was achieved by constant circulation for more than 24 h using a peristaltic pump system. Bottle filling took place in a clean room. The (conditioned) 500 mL polyethylene bottles were filled with the CRM solutions by means of a peristaltic pump. The handling of the sampling bottles during the filling process and the filling of the bottles were carried out in Clean Room atmosphere. After bottling the bottles were labelled and put in two zipper bags in the Clean Room. The bottles were then taken out of the Clean Room, and each bottle was covered with a layer of bubble plastic and put in boxes designed to fit to the bottles, thus minimising movement of the bottle in the box. This was done to achieve maximum protection during transport. The boxes were finally closed loosely with an elastic band to enable a final inspection before distribution.

TABLE 8.15

SUBSTANCES ADDED FOR THE PREPARATION OF THE CANDIDATE CRMS
AND CONCENTRATIONS EXPECTED UPON SPIKING

Element	CRM 609 ($\mu\text{g kg}^{-1}$)		CRM 610 ($\mu\text{g kg}^{-1}$)	
	Before spiking	Amount added	Before spiking	Amount added
Al	0.2	47	0.3	155
As	<0.5	0.940	<0.5	10.1
Cd	0.05	0.118	<0.005	3.10
Cu	2.0	2.0	<0.5	46.6
Ni	3.0	6.27	<0.1	23.3
Pb	<0.05	1.57	<0.005	7.76

8.6.3.2. CRMs for bromide

Two new 200 L polyethylene containers were cleaned by rinsing three times with tap water (that had been running for five minutes). Two other new 200 L polyethylene containers to be used for the collection of filtered samples were rinsed with tap water followed by rinsing with filtered (0.45 µm membrane filter) tap water. Tubes were soaked in Milli-Q water prior to use, for at least 24 h.

The filters used for sample filtration were a Sartorius GF 0.2 µm Sartopure pre-filter cartridge followed by a Sartorius Sartobran PH membrane filter capsule (particle retention size 0.45 µm). The filters were autoclaved after used. Filters were used until clogging occurred.

For the CRM 611 (low level), 150 L aerated groundwater was collected from Helsingør waterworks. The sample was aerated for one day, then filtered with the above mentioned filtration system and pumped into a clean container. 70 kg was weighed and mixed with 70 kg freshly distilled water cooled to 25°C before use. The water was mixed for 15 min with a mechanical stirrer unit, after which a sample was taken for a pre-contamination microbiological test. No microbiological growth was found in the sample. The sample was then re-filtered through a Sartobran filter (0.45/0.2 µm pore size). Samples were filled in 25 mL ampoules with an ampoule-filling machine. The first and last ampoules filled were tested for microbiological contamination and no signs of microbiological contamination were detected. The filling procedure lasted 4 h. Ampoules were autoclaved at 120°C for 20 min; they were then labelled individually and sets of four ampoules were packed in specially designed boxes to which individual numbers were given (the four ampoules in each box were given the same number of the respective box).

For the CRM 612 (high level), 200 L aerated groundwater was collected from Køge waterworks. Similar preparation procedures were used in comparison with CRM 611, with the exception that this material was not diluted with distilled water.

The analytical methods used to verify the between-ampoule homogeneity were ETAAS (Al, Cd, Cu, Pb and Ni) and HGAAS (As). No inhomogeneities could be detected for any of these elements in both CRMs [17]. For bromide, determinations were carried out by ion chromatography; the materials were both found to be homogenous [18].

The stability tests were performed over 52 weeks storage at +4°C and +20°C. A short-term stability study was also carried out at +40°C to test worst-case transport conditions. No instability could be detected at any of the conditions tested [17,18] in all the materials.

8.6.4. Certification

Various analytical techniques were used in the certification, involving different types of pretreatment. The methods of final determination are summarised in Table 8.16.

The most common sources of systematic errors that were detected during the technical discussions were contamination problems, calibration errors or uncontrolled matrix effects. Results obtained by TXRF could not be retained for certification since this

TABLE 8.16

SUMMARY OF THE TECHNIQUES OF FINAL DETERMINATION (CRMS 609–610)

Element	Techniques
Al	ETAAS, HR-ICPMS, ICP-AES, ICPMS, INAA
As	ETAAS, HGAAS, HR-ICPMS, ICPMS, RNAA
Cd	DPASV, ETAAS, HR-ICPMS, ICPMS, ID-ICPMS
Cu	DPASV, ETAAS, HR-ICPMS, ICPMS, ID-ICPMS
Pb	DPASV, ETAAS, HR-ICPMS, ICPMS, ID-ICPMS

technique does not comply with traceability requirements requested for certification; however, no doubts were expressed on its suitability for routine measurements and the results were used as confirmation for As, Cu and Pb.

For aluminium, the lack of use of modifier for Al determination by ETAAS explained low results which were consequently withdrawn.

High results obtained by one laboratory for arsenic by ICPMS were likely due to ArCl-interferences; the results were withdrawn. This interference was confirmed by another laboratory which tested the use of silver nitrate cartridge to remove chloride: while no difference was observed for CRM 609 between filtered and not filtered samples, significant differences were observed for CRM 610 which contain higher chlorine content (i.e. higher results were obtained for not filtered samples). A precipitate was observed by another laboratory in the CRM 610 which was attributed to the presence of higher Fe content in comparison to CRM 609. Digestion by high-pressure ashing was necessary to ensure a good recovery for As by DPASV.

In the case of lead, it was recommended that calibration be performed by standard additions to take matrix effects into account. Similar problems of precipitation in CRM 610 were experienced by one laboratory, which withdrew its results obtained by HR-ICPMS, DPASV and ETAAS.

A wide spread of results was observed for Ni in both CRMs. After technical scrutiny, it was found that most of the outlying results were due to either calibration errors (using external calibration) or blank problems. A strong recommendation was given to use standard additions for the determination of Ni by ETAAS in these materials; this recommendation was supported by the fact that sets of data of laboratories which applied standard additions were in good agreement (using the following techniques: ICPMS (2 laboratories), ETAAS, and ID-ICPMS). In the case of CRM 610, high results obtained by ICPMS by one laboratory could be due to interferences on ^{58}Ni from ArO and NaCl which were only observed to a lesser extent on CRM 609. Considering the spread of results, it was decided to consider nickel as indicative only on the basis of the data obtained by standard additions.

Bromide was determined solely by ion chromatography, whereas total bromine was determined by ICPMS and INAA. Bromide was hence certified on the basis of IC measurements, whereas total Br was given as certified value.

The large spread of results could not allow the certification of iodide in any of the CRMs; consequently, indicative values were proposed only [18].

The certified values, along with their uncertainties, are given in Table 8.17.

8.6.5. Participating laboratories

The preparation of the reference materials and the verification of their homogeneity and stability were carried out by the Water Quality Institute (VKI) in Hørsholm (Denmark) and KIWA N.V. in Nieuwegein (The Netherlands). The following laboratories participated in the certification campaign: Anglian Water Services Ltd., Cambridge (United Kingdom); Aristotle University, Lab. of Analytical Chemistry, Thessaloniki (Greece); A/S AnalyCen, Fredericia (Denmark); Bundesamt für Seeschifffahrt und Hydrographie, Hamburg (Germany); Canal Isabel II, Madrid (Spain); CNRS, Service Central d'Analyse, Vernaison (France); Empresa Portuguesa das Águas Livres, Lisbon (Portugal); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Finland Miljöcentral, Helsinki (Finland); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Istituto Superiore di Sanità, Roma (Italy); Institut Scientifique de Service Public, Liège (Belgium); KIWA N.V., Nieuwegein (The Netherlands); Ministère de l'Intérieur, DGCCRF, Talence (France); NIVA, Oslo (Norway); NV-PWN Waterleidingbedrijf Noord-Holland (The Netherlands); State Laboratory, Dublin (Ireland); Stockholm Universitet, ITM, Solna (Sweden); Sociedad General das Aguas de Barcelona (Spain); Universiteit Gent, I.N.W., Gent (Belgium);

TABLE 8.17A

CERTIFIED VALUES FOR CRMS 609 AND 610

p: sets of results (each of at least 6 replicates)

CRM 609	Certified value in $\mu\text{g kg}^{-1}$	Uncertainty in $\mu\text{g kg}^{-1}$	p
Al	47.7	1.6	14
As	1.20	0.12	8
Cd	0.164	0.012	16
Cu	2.48	0.09	9
Pb	1.63	0.04	10
CRM 610	Certified value in $\mu\text{g kg}^{-1}$	Uncertainty in $\mu\text{g kg}^{-1}$	p
Al	159	4	12
As	10.8	0.4	10
Cd	2.94	0.08	13
Cu	45.7	1.5	7
Pb	7.78	0.13	9

TABLE 8.17B

CERTIFIED VALUES FOR CRMS 611 AND 612

p: sets of results (each of at least 6 replicates)

Bromide	Certified value in $\mu\text{g kg}^{-1}$	Uncertainty in $\mu\text{g kg}^{-1}$	p
CRM 611	93.4	3.9	10
CRM 612	252	10	8

Water Quality Institute, VKI, Hørsholm (Denmark); Water Research Centre, Medmenham (United Kingdom)

8.7. TRACE ELEMENTS IN ESTUARINE WATER

8.7.1. Introduction

National and international marine monitoring programmes have been initiated worldwide to assess the quality of the marine environment, e.g. the Joint Monitoring Programme of the Oslo/Paris Commissions. Similar programmes exist to monitor the quality of freshwater environments e.g. the Rhine river monitoring programme. As said at various occasions in this book, it is essential in monitoring programmes to ensure that the data produced are of sufficient accuracy so that results of different laboratories obtained over a number of years can be compared. With respect to estuarine water analysis, improvements were found to be necessary which resulted from an interlaboratory study organised by the BCR in 1989 in which specific analytical problems have been encountered in comparison to coastal and offshore seawater [19]. In addition to the relatively low concentrations of trace elements and the salt matrix, the dissolved organic matter (DOM) is usually much higher than in open seawater. As a result of strong complexing behaviour due to this DOM, total trace metal determinations in estuarine water may fail to perform quantitatively. For instance when methods involve complexation/extraction techniques (as in AAS procedures) or electrochemical measurements (e.g. DPASV) an underestimation of the concentration may result. Certified reference materials are necessary to verify whether their method of preference would be applicable to estuarine waters. However, the preparation of suitable estuarine water materials is difficult due to the possible formation of suspended matter following flocculation of colloids. The only CRM available in 1992 for the quality control of trace element determinations in estuarine water had been prepared by the National Research Council of Canada in 1991 [20]. This material was, however, prepared from relatively pristine water with a low DOM content. In view of the specific difficulties of analysis of high DOM containing estuarine water, the BCR decided to organise a series of interlaboratory studies to establish and possibly to improve the state of the art of trace element determinations in estuarine water in Europe. As a follow-up a CRM has been produced (CRM 505) which has been certified for its content in Cd, Cu, Ni and Zn [21,22].

8.7.2. Feasibility study

Owing to the difficulty of preparing candidate reference materials of estuarine water and the analytical difficulties encountered by the laboratories for trace element determinations, it was decided to carry out preliminary investigations to optimise the sampling and sample pretreatment procedures, and to evaluate the state of the art of estuarine water analysis in Europe. A limited batch of estuarine water sample was therefore collected for this purpose.

All the materials used for the sample collection were made of either Linear Polyethylene (LPE, 1 litre bottles, storage container) or teflon tubing, with the exception of a short length of silicone tubing (C-flex) in the head of the peristaltic pump. The materials were carefully cleaned according to procedures commonly used for trace metal analyses in seawater [23]:

- (a) washing with demineralised water to remove dust and particulate polymer remnants;
- (b) leaching with reagent grade nitric acid (2 volumes of acid in 3 volumes of water) for more than 5 days (1L bottles) or 3×5 days (3000 L tank). In this cleaning procedures, as well as in the following steps, all bottles were filled to the neck;
- (c) leaching with diluted HNO_3 p.a. (1:12) for more than 5 days;
- (d) filling with ultrapure water acidified to $\text{pH} = 1.6$ (with HNO_3 , highest purity; sub-boiling distilled); this solution was kept in the bottles and tank until their use.

All operations (cleaning, filtration, acidification and sample handling) were carried out in a specially designed Class 100 room, either fitted in a standard 20 ft transport container which was fully equipped as a chemical laboratory or in a room transformed as such on board of the research ship. All operations with the sample were performed in a closed system, except for the sample acidification and the filling of the bottles, which were performed in a laminar flow clean bench inside the clean room. All personnel working in the clean area used polythene gloves, dust-free garments and shoe-covers.

For cleaning, the tank was filled to such a level that by rolling the tank over the different sides, all sides were soaked with acid. For the final cleaning step the tank was filled up to the top with ultrapure water acidified with HNO_3 . The used bottles (LPE) had a capacity of 1 L and were closed by a cleaned LPE screwcap. All care was taken to minimise contamination (operations carried out in a clean room, use of clean benches).

8.7.3. Interlaboratory studies

Two interlaboratory studies were organised, using samples collected in the Tagus Estuary (Portugal) in 1990 and 1992, respectively [19]. Locations with salinities of around 8 ‰ and 16 ‰ were selected at two different sites; the collection was carried out from a rubber boat moored along side the ship to minimize contamination from the hull; teflon tubing was used for transferring the samples in a closed system into a 400 L storage tank.

The samples were filtered through an in-line 0.45 μm filter cartridge placed after the pump, acidified to pH of ca. 2 by addition of HCl to the storage container after homogenization. The bottles were filled in a clean bench using the peristaltic pump. Care was taken to perform the filling in one continuous operation to avoid a prolonged

stay of the water sample in the tubing. The bottles were packed in two polythene self-seal bags and stored at ambient temperature. Bottles were randomly selected during the bottling procedure for homogeneity and stability studies. Additional details are given elsewhere [19].

In open seawater the content of organics is low. Therefore, laboratories usually analysing seawater take only modest precautions or do not take measures at all for any complexation risks. Estuarine water, however, contains a higher content of organic matter and is therefore more prone to interferences. This explains why many laboratories who previously had good results in seawater analysis made errors when analysing estuarine water.

For an accurate analysis of estuarine water a good breakdown of organics is necessary. Owing to the high organic matter content, low results could be expected due to an incomplete extraction (scavenging of elements by complexing ligands present in the water and consequent competition with the added complexant). It was therefore recommended to apply a destruction step prior to the extraction, to determine the yield of the extraction (e.g. for Cd and Cu), to use an excess of extractant or to apply a multiple step extraction and to add the acid for the back extraction in its concentrated form in order to break down the complex (the solution should then be diluted after half an hour). The determination of the extraction yields may be done using reference materials (which are still hardly available) or by spiking an estuarine water with analytes, allowing to reach equilibrium and performing the extraction.

In the case of e.g. voltammetric techniques, the high content of organic matter could lead to erroneous (too high) results even in case standard addition procedures are applied.

UV irradiation or even a digestion using nitric acid may appear necessary especially in the case of organic species as observed in the case of arsenic which can be present as very stable species (arseno-betaine and -choline). Nickel is another example of a strongly bound metal.

Table 8.18 summarises the results obtained in the first interlaboratory study. From this table, it is clear that improvements were strongly required in the trace element determinations in estuarine water, particularly for Cd, Co, Mn, Pb and Zn. A comparison with the CVs obtained in the certification of trace elements in seawater [24] also highlighted the enhanced difficulties in the analysis of estuarine water; these CVs ranged from 9.5% (Cu) to 21.4% (Pb) for concentrations three times lower than that found in the estuarine water samples.

Table 8.19 gives a summary of the results obtained in the second interlaboratory study. Specific remarks were made for some elements:

For cadmium, one participant remarked that without using standard additions, only 50% of the Cd content was recovered. A strong requirement is therefore to perform calibration by standard additions for estuarine water analysis. As mentioned earlier, the participants stressed that the extraction efficiency has to be verified, particularly for Cd, as it depends on the content of complexing agents present in estuarine water matrices. The risk of incomplete extraction should be checked by repeated extractions. The problem raised by the presence of organic matter may be overcome in many cases by the use of UV irradiation or addition of acids. This possible pretreatment should

TABLE 8.18

SUMMARY OF THE RESULTS OF THE FIRST INTERLABORATORY STUDY. THE RESULTS ARE GIVEN IN nmol kg^{-1}

SAMPLE A

Element	Number of sets	Number of sets accepted	Mean \pm s.d. (accepted sets) nmol kg^{-1}	CV (%) raw data	CV (%) accepted data
As	3	3	63.7 ± 2.3	3.5	3.5
Cd	17	12	0.92 ± 0.18	172	20.0
Co	5	1	0.80 ± 0.10	43.7	12.6
Cu	19	14	31.6 ± 4.2	58.3	13.1
Fe					
1st filt.	8	3	17.9 ± 1.8	165	10.0
2nd filt.	3	1	16.5 ± 2.5	61.5	15.3
Pb	12	3	0.37 ± 0.05	227	14.1
Mn					
1st filt.	6	3	11.9 ± 0.7	57.0	5.7
2nd filt.	3	3	10.4 ± 1.4	13.3	13.3
Ni	13	11	17.0 ± 2.4	70.6	14.0
Zn	15	11	140 ± 28	55.0	20.3

SAMPLE B

Element	Number of sets	Number of sets accepted	Mean \pm s.d. (accepted sets) nmol kg^{-1}	CV (%) raw data	CV (%) accepted data
As	3	3	51.0 ± 3.4	6.7	6.7
Cd	16	11	0.64 ± 0.16	237	25.3
Co	5	2	0.66 ± 0.15	65.0	22.1
Cu	17	12	36.1 ± 2.3	146	6.5
Fe					
1st filt.	7	2	41.1 ± 0.3	132	0.7
2nd filt.	3	1	39.7 ± 3.5	52.6	8.8
Pb	10	4	0.30 ± 0.11	202	38.6
Mn					
1st filt.	5	3	11.2 ± 1.6	41.2	14.1
2nd filt.	3	3	11.1 ± 2.2	19.9	19.9
Ni	13	10	23.5 ± 3.0	70.8	12.9
Zn	14	11	134 ± 28	209	21.0

be carefully investigated prior to analysing the sample. The participants using DPASV also found relatively low concentrations which allowed to suspect a strong effect of organic compounds which were not completely destroyed by UV irradiation (e.g. organic

TABLE 8.19

SUMMARY OF THE RESULTS OF THE SECOND INTERLABORATORY STUDY.
THE RESULTS ARE GIVEN IN nmol kg^{-1}

Element	Number of sets	Number of sets accepted	Mean \pm s.d. (accepted sets) nmol kg^{-1}	CV (%) raw data	CV (%) accepted data
Cd	18	12	0.26 ± 0.03	69.0	11.5
Co	4	—	—	172	—
Cu	21	17	41.5 ± 3.1	23.3	7.5
Fe	10	7	32.0 ± 5.1	125	15.9
Pb	10	7	0.52 ± 0.08	81.7	15.4
Mn	7	—	—	63.9	—
Ni	16	12	24.3 ± 2.6	78.0	10.7
Zn	6	7	81.3 ± 8.9	30.8	10.9

compounds originating from agricultural waste). Digestion by complete evaporation until dryness of the sample or addition of inorganic acid before UV irradiation was recommended, e.g. adding different acids in order to optimise the technique prior to certification; the addition of H_2O_2 was strongly suggested. Finally, as mercury arc tubes decrease in efficiency with time, the use of a new UV lamp to ensure a complete destruction of organic materials was advised.

In the case of copper, spectral interferences in ICPMS were found, leading to high results which were probably caused by a spectral overlap from an unidentified polyatomic species at mass 65. The existence of this species was confirmed by a mass spectrum obtained by high resolution ICPMS, although its exact composition could not be determined. DPASV and CSV were in good agreement with the other techniques in this case. Provided that a proper destruction of organic matter be carried out, no problems were actually suspected for Cu.

For Iron, FAAS results were suspected to be too close to the determination limits. Higher volumes should be used or a preconcentration step should be carried out. One participant reanalyzed the sample after 4-times dilution and found a value closer to the mean (35 nmol kg^{-1}). It was suspected that the too high value was out from the linear range and was hence overestimated. Problems with the low pH were experienced and the participant recommended that more ammonia be used for neutralisation. Such a warning should be given at the certification stage. Certification could be contemplated providing that additional techniques are used, e.g. ICPAES.

Manganese on chelating resin depends on its oxidation state. One participant checked the recovery by adding Mn and found a recovery of 95%. It was suggested, however, to reduce all the Mn with e.g. SnCl_2 . Problems could be linked to the behaviour of different Mn-species. The participants agreed that more investigations should be carried out on this element before contemplating certification.

FAAS was not considered to be a suitable technique for the determination of nickel at this level of concentration. This technique could possibly be considered in a certification

providing that careful quality control schemes are applied and that the methods are verified with relevant CRMs.

The results of the second interlaboratory study clearly showed that many laboratories had still difficulties in analysing estuarine water samples. The Table 8.19 indeed illustrates the very high coefficient of variation (CV) obtained between laboratories for the raw data. However, after the technical discussion, most of the systematic errors that occurred in the outlying results could be explained and the CVs of the accepted sets of data were much more acceptable, ranging from 7.5 to 15.9%. The participants agreed that this range of CVs for Cd, Cu, Fe, Pb, Ni and Zn was corresponding to the state of the art of estuarine water analysis and, consequently, recommended the organisation of a certification campaign. Only Co and Mn were still questionable.

A comparison with the results obtained in the first interlaboratory study (Table 8.18) showed a clear improvement in the quality of the determination of most of the elements, particularly cadmium (considering the much lower concentrations determined in the second exercise), copper, lead and zinc. For iron and nickel, the CVs between laboratories were approximately the same.

8.7.4. Production of the candidate CRM

The sampling equipment, cleaning procedures and precautions taken at the sample collection stage were similar to the ones described in the previous paragraph. A tank of 3000 L was used for the storage of the candidate reference material immediately after collection. The sampling cruise was carried out on board of the N.R.P. AURIGA of the Hydrographical Institute of the Portuguese Navy on 8–9 May 1992 [21]. The sampling followed a survey of salinity and suspended matter gradients to optimise the time and location of the actual collection. Three high-tide periods were necessary to collect all the required material which was performed during 2 h around high tide each time at a salinity of ca. 12 ‰. The salinity was monitored with a probe connected to the sampling device and the ship moved to follow, as much as possible, about a constant salinity.

The first 30 L of the collected estuarine water were discarded and then the water was collected directly in the tank after filtration (as described above). Due to clogging, the filters had to be replaced several times during the procedure. Each new filter was flushed with several litres of sample before the tubing was connected again to the storage container. The candidate CRM was then homogenised as described above.

The initial pH of the homogenised sample was 7.7 which was adjusted to 1.7 by addition of hydrochloric acid (Supra Pur, Merck) directly in the tank. The sample was further homogenised by circulating the water for one hour every six hours throughout the storage period, using a metal free magnetic pump. To allow colloidal material to flocculate, the sample was stored for 9 months before further treatment.

To check for possible contamination that might have occurred during sampling, samples were collected in the clean room, directly from the sampling tube after filtration. Tests were made on Cd, Co, Cu, Ni and Zn determined by ETAAS after APDC/DDDC complexation (Cd, Cu) or DPASV after UV-irradiation (Cd, Co, Cu, Ni and Zn).

Samples collected at three different occasions were analysed and no significant differences could be observed between the results obtained for the different elements (variations were within the uncertainty of the methods used).

In order to remove the aggregate formed during storage of the sample, a second (cleaned) tank of 2000 L was used for further treatment of the candidate CRM. A Teflon tube was connected between the two tanks and the estuarine water was passed from the first tank (3000 L) to the second tank through a 0.45 µm membrane in-line filter placed in a laminar flow cabinet. After filtration, the water was circulated overnight (using a pump as described previously) to ensure a good homogenisation before bottling. It is stressed that the different treatments applied to the sample may have led to a material easier to analyse in comparison with natural samples; this compromise was necessary to ensure that the homogeneity and stability were suitable for this material to be used as a candidate CRM.

Analyses were carried out prior to bottling to verify that no contamination had occurred during the second filtration step.

The bottling and storage procedures followed those previously tested in the preliminary investigations (see section on background of the project). Acidified suprapure water contained in the LPE bottles was discarded. The volume of soaking liquid which remained in the bottles was observed to be minimal and therefore no rinsing step with sample was considered to be necessary. The bottles were filled directly using the peristaltic pump and closed with LPE caps.

Each bottle was packed in two zip-bags and identified on the outer bag which was sealed. The bottles were then packed in cardboard boxes and stored at ambient temperature prior to shipment to the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium).

The between-bottle homogeneity of the candidate CRM was verified by the determination of Cd, Cu and Zn, using the same analytical methods described in the paragraph on preliminary investigations. No significant differences were observed between the CV of the method and the CV between-bottles [23] and the material was considered to be homogeneous as was demonstrated at the level of 10 g and above.

The stability of Cd, Cu and Zn was tested at ambient temperature over a period of 12 months. Determinations were performed at least in three-fold using the procedures detailed above. No instability could be demonstrated and the material was considered suitable for certification.

8.7.5. Certification

Each laboratory which participated in the certification made a minimum of five independent replicate determinations. The techniques used by the participating laboratories are summarised in Table 8.20; they were based on different pretreatment procedures (e.g. complexation/extraction for AAS, UV irradiation for ASV) and detection techniques (e.g. ETAAS, ICPAES, ICPMS, DPASV).

The comments reported below are a result of the technical scrutiny of the results which enabled sources of discrepancy to be identified for some elements.

Following the recommendations of the previous interlaboratory studies, the

TABLE 8.20

SUMMARY OF THE TECHNIQUES USED IN THE CERTIFICATION OF CRM 505

Element	Techniques
Cd	DPASV, ETAAS, ICPMS, ZETAAS
Cu	DPASV, DPCSV, ETAAS, ICPMS, ZETAAS
Ni	DPCSV, ETAAS, ICPAES, ICPMS, TXRF, ZETAAS
Zn	DPASV, ETAAS, FAAS, ICPAES, ICPMS, ZETAAS

laboratories using DPASV carefully verified the destruction of organic complexes. For example, one laboratory applied UV irradiation for 7 h, followed by an addition of H_2O_2 and further irradiation for 7 h. If the hydrogen peroxide addition would be performed in the first place (i.e. before the UV treatment) the reagent would be decomposed which highlights the need to start by an UV irradiation step. Another laboratory submitted two sets of results obtained by DPASV. One set was obtained after UV irradiation and further digestion (mean of $0.713 \pm 0.039 \text{ nmol kg}^{-1}$) and a second set involving H_2O_2 digestion (mean of $0.774 \pm 0.050 \text{ nmol kg}^{-1}$). The higher value obtained with the hydrogen peroxide treatment showed that a digestion step was necessary to obtain a good recovery of cadmium in this matrix, which confirmed the discussions of results of the second interlaboratory exercise. The second set was accepted for certification.

The spread of results obtained for cobalt did not allow any conclusion to be drawn, except that this element is difficult to be determined in such type of sample as it is strongly bound to the matrix. The mean of laboratory means is given as indicative value in the certification report [21].

Low recoveries were observed in some instances for copper (verified with the CASS-2 CRM) which justified results to be withdrawn.

The low Pb levels, associated to the high level of particulate matter in the original estuarine water sample rendered the determination of this element too difficult for allowing certification. The laboratories stressed that contamination and blank problems hampered a good agreement to be obtained which outlines that the state of the art is rather poor for such matrix. A new mean was calculated and is given as indicative value in the certification report [21].

The manganese results were separated in two groups of data. Doubts were expressed on the separation (performed on chelating resin) due to the presence of different Mn-species (oxidised and complexed forms). An indicative value is given for this element in the certification report [21].

The certified values for Cd, Cu, Ni and Zn are presented in Table 8.21 along with their uncertainty.

Table 8.22 presents the results of the comparison of different methods used in this certification. The CVs within one method are generally of the same order of magnitude as the CVs between different techniques. For ICPMS (cadmium), ETAAS (nickel) and FAAS (zinc) the larger CVs are due to operating close to the limits of determination;

TABLE 8.21

CERTIFIED VALUES FOR CRM 505 (IN nmol kg⁻¹ AND mg kg⁻¹)

p: sets of results (each of at least 5 replicates)

Element	Certified value (nmol kg ⁻¹)	Certified value (µg kg ⁻¹)	P
Cd	0.80 ± 0.04	0.090 ± 0.005	12
Cu	29.4 ± 1.5	1.87 ± 0.10	12
Ni	24.1 ± 2.0	1.41 ± 0.12	10
Zn	172 ± 11	11.2 ± 0.8	15

TABLE 8.22

RESULTS OF THE EVALUATION OF CONSISTENCY OF THE METHODS USED

Element	Techn. of final determination	CV (%) between means of lab. with the same technique	CV (%) between means of diff. techniques	Nr of sets of results
Cadmium	ETAAS	4.5	5.5	6
	ICPMS	11.9		2
	DPASV	5.1		3
Copper	ETAAS	7.6	7.2	7
	DPASV	5.2		3
Nickel	ETAAS	10.7	10.9	4
	ICPAES	2.8		2
	DPCSV	0.8		2
Zinc	ETAAS	6.4	10.1	2
	FAAS	12.3		6
	ICPAES	5.4		2
	DPASV	6.3		3

since the techniques were shown to be under control at this level of concentrations, which was hence found acceptable for certification.

8.7.6. Participating laboratories

The preparation of the estuarine water samples was performed in collaboration between the Instituto Hidrográfico in Lisbon (Portugal), the Laboratory for Applied Marine Research (IMW-TNO) in Den Helder (The Netherlands) and the Labor für Spurenanalytik in Bonn (Germany). The homogeneity and stability studies were carried out by a consortium of laboratories, namely the Biologische Anstalt Helgoland in Hamburg (Germany), the Instituto Hidrográfico in Lisbon (Portugal), the Labor für Spurenanalytik in Bonn (Germany) and the Rijkswaterstaat (Dienst Getijdewateren)

in Haren (The Netherlands). The following laboratories participated in the certification campaign. The participants are gratefully acknowledged for their contribution to this certification campaign: Aristotelian University, Thessaloniki (Greece); Biologische Anstalt Helgoland, Hamburg (Germany); Bundesamt für Seeschifffahrt und Hydrographie, Hamburg (Germany); Chalmers University, AMK, Göteborg (Sweden); Forschungszentrum für Umwelt und Gesundheit, Neuherberg (Germany); IFREMER, Nantes (France); IMW-TNO, Lab. Toegepast Marien Onderzoek, Delft (The Netherlands); Instituto Hidrográfico, Lisboa (Portugal); Kemira Denmark A/S, Vedbaek (Denmark); Labor für Spurenanalytik, Bonn (Germany); Nederlands Instituut voor Onderzoek der Zee, Den Burg (The Netherlands); Rijkswaterstaat, Dienst Getijdewateren, Haren (The Netherlands); Studiecentrum voor Kernenergie, Mol (Belgium); Universitaire Instelling Antwerpen, Wilrijk (Belgium); University of Liverpool, Oceanography Laboratory, Liverpool (United Kingdom); Universidad Nova, Lisboa (Portugal); Water Quality institute, Hørsholm (Denmark).

8.8. TRACE ELEMENTS IN SEAWATER

8.8.1. Introduction

Many directives have been implemented by the Council of the European Communities regarding the quality of the marine environment, for example to protect sea life in general, to prevent damage by spills etc. As an indication of international concern, several conferences have been held which have led to the adoption of several conventions, e.g. the Oslo-London dumping convention, the Paris convention dealing with pollution from land based sources, and the Barcelona convention dealing with the pollution from the Mediterranean Sea. The quality of the marine environment is being monitored by analysing different matrices such as water, sediment and/or biota. With respect to water, monitoring is often hampered by the high risks of contamination that may occur either during sampling or sample pretreatment and analysis, owing to the low levels of contaminants in this medium. CRMs of seawater are therefore of paramount importance for verifying the quality control of e.g. inorganic analysis and the BCR has developed a material in 1989, CRM 403, which was certified for its contents of Cd, Cu, Pb, Mo, Ni and Zn, following a series of interlaboratory studies [24,25].

8.8.2. Interlaboratory studies

Two interlaboratory studies were organised prior to the certification campaign. The first one dealt with the analysis of artificial seawater and the second exercise concerned the analyses of natural and spiked seawater. The results obtained for Cd, Cu, Pb and Zn are compared in Table 8.23. The CV between all the laboratories appeared to be quite high in the second round-robin exercise. However, the participation in such intercomparison combined with critical discussions of methods and results was found to be a most useful tool in obtaining a high level of accuracy (which is reflected in the

TABLE 8.23

CVS OF THE MEASUREMENTS OBTAINED BETWEEN THE LABORATORIES IN THE INTERCOMPARISONS AND CERTIFICATION (CONCENTRATIONS GIVEN IN nmol kg⁻¹)

Element	First intercomparison		Second intercomparison				Certification	
	Artificial seawater		Spiked seawater		Natural seawater		CRM 403	
	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
Cd	79.2±25.1	31.7	7.60±1.83	24.1	0.19±0.07	35.2	0.175±0.018	10.3
Cu	142±21	14.6	19.3±5.2	26.6	8.75±2.14	24.5	3.90±0.37	9.5
Pb	-	-	4.35±2.00	46.0	0.57±0.83	146.0	0.117±0.025	21.4
Zn	145±28	19.1	26.4±10.9	41.3	16.6±7.4	44.5	25.7±2.9	11.3

CVs obtained in the certification stage). Only those laboratories that demonstrated a high level of accuracy were invited to participate in the certification.

8.8.3. Production of the reference material

In order to minimise the contamination, all materials which came into contact with the seawater either during sampling at sea or storage were made of either Linear Polyethylene (LPE) for bottles and tanks or Teflon (FEP) for tubing, with the exception of a short length of silicone tubing (C-flex) in the head of the peristaltic pump. For sampling, a polythene cable was lowered with a PVC-coated concrete weight. Starting about 1.5 m above this weight, Teflon tubing (12×14 mm) was attached to the cable with cable ties. The tubing (total length of about 35 m) was connected to a peristaltic pump with a maximum capacity of about 10 L min⁻¹.

The mixing and storage tank was a 3000 L LPE container of which the zinc-coated steel reinforcements were covered with plastic prior to use; the tank was stored on the ship in a special transport container. FEP tubing connected the pump, filter and the tank in a closed system. The bottles used had a capacity of 2 L and were closed by a cleaned LPE insert and screwcap. The insert allowed to prevent any leakage from the bottles. The cleaning procedures, storage tank, and tubing, was based on earlier work [21]. The sequential cleaning steps carried out at ambient temperature involved:

- (1) washing with demineralised water to remove dust and particulate polymer remnants;
- (2) leaching with reagent grade nitric acid (Baker, 2:3 v:v) for more than 5 days (2 L bottles) or 3×5 days (3000 L tank); in this cleaning procedure as well as in the following steps, all bottles were filled to the neck;
- (3) leaching with HNO₃ (Instra analysed, Baker, 1:12 v:v) for more than 5 days;
- (4) filling with Milli-Q water acidified to pH 1.6 (with HNO₃, Supra pure, Merck); this solution was kept in the bottles and tank until their use.

The 3000 L tank was filled to such a level that by rolling the tank over to the different sides, all sides were soaked with acid. For the final step the tank was filled up to the top with Milli-Q water acidified with pH=1.6 (HNO₃, Supra pure, Merck). All operations

(cleaning, filtration, acidification, sample handling) were carried out in an especially designed Class 100 clean room fitted in a standard 6 m transport container which was fully equipped as a chemical laboratory. The container was used both on the ship and on the shore. All operations with the seawater sample were carried out in a closed system except for the sample acidification and the filling of the bottles, which were performed on a laminar flow clean bench inside the clean room container. All personnel working in the container used polythene gloves, dust-free garments, and shoe-covers.

The sample collection was performed on 20–21 March 1988 in the central part of the Southern Bight of the North Sea. The ship was kept drifting during the sampling; to stay away from the hull and from the bottom sediment, it was decided to collect the sample at a depth of 20 m. Investigation on the lateral and vertical composition variations had revealed that the water body was homogeneous within narrow limits.

The characteristics of the water body sampled were: water depth: 37–49 m; salinity: 34.61–34.75 ‰; pH: 7.87–8.01; temperature: 7.7°C. Approximately 2600 L of seawater were collected directly in the cleaned tank. The sample was passed on line over a 0.45 µm membrane filter (versaflo capsule, Gelman) which was positioned after the pump. Although no serious clogging of the filter occurred, it was replaced twice during the sampling operation. After each filter installation, about 20 L of sample were discarded.

A preliminary experiment showed that the addition of 2 mL of nitric acid to 1 L of seawater resulted in a pH of 1.5. Therefore, 6 L of this acid (Supra pure, Merck) were added to the batch of collected seawater after about half of the total volume had been sampled.

As the seawater was collected from one water body whilst the ship was slowly drifting and because of the ship's movements and the constant mixing with the incoming flux of seawater, the content of the tank was assumed to be homogeneous. Nevertheless, the seawater was circulated in the tank using the pump during 24 h prior to the bottling which was performed on the shore. The acidified Milli-Q water (pH 1.6) contained in the bottles was discarded and the bottles were filled directly from the tank using the peristaltic pump. The filling was performed in one continuous operation. The bottles were then packed in two polythene zip-bags; the outer bag was identified by a label and sealed. The bottles are stored at ambient temperature.

The between-bottle homogeneity was verified by the determination of Cd, Cu, Pb and Zn on one intake of 50 g. Cd and Pb were determined by ZETAAS whereas Cu and Zn were determined by DPASV. The results showed that the material is homogeneous at the 50 g level and above [25].

Common oceanographic experience [26] indicates that the content of trace metals does not change in time if the samples are filtered and acidified to a pH value below 1.6, as was done in the preparation of the material. All bottles were kept in closed cardboard boxes at ambient temperature over a period of 12 months. Cd, Pb, Cu and Zn were determined at the beginning of the storage period and after periods of 6 and 12 months. The long-term stability was established for Cu and Zn after storage of an extra 12 months under the same conditions. The results of the tests did not reveal any signs of instability. In addition to the stability tests described before, a test simulating transport at elevated temperature was performed. The bottles were stored in the dark at a temperature of 40°C for a period of 6 weeks. Analyses were analysed in duplicate

using DPSAV for the elements Zn, Cd, Pb and Cu after intervals of 1 and 2 weeks and at the end of the 6 weeks period. Results were compared with those obtained after 1 day. No significant changes in element concentrations could be observed even after a period of 6 weeks at elevated temperature.

8.8.4. Certification

Eighteen laboratories from seven European countries participated in the certification. The techniques of final determination used by these laboratories for the different elements is summarised in Table 8.24.

The certified values of Cd, Cu, Mo, Ni, Pb and Zn are presented in Table 8.25 along with their calculated uncertainties.

The comparison of results per technique did not allow us to detect any bias which could be attributed to one particular technique. As shown in Table 8.26, the CVs within one method are systematically larger, or of the same order of magnitude (Ni) than those between different techniques. Consequently, it cannot be inferred that the results of one technique do not agree with those of other techniques for the elements mentioned.

8.8.5. Some remarks on the analysis of the material

It is emphasised that the CRM 403 is a seawater sample that has already been treated (filtration, acidification) to enable storage. Hence, analytical results obtained that match the certified values do not give information on the sample handling prior to the chemical analysis. The handling, e.g. sampling, filtration, acidification, storage etc. of real samples should be performed with the necessary care to preserve their original state.

Seawater contains dissolved organic matter that may complex trace metals. The possible effects in the analysis depend on the method of analysis and the element itself. In methods using a complexation step (AAS, ADPCSV) the naturally present ligand may inhibit the complexation reaction or part of it, thus causing an underestimation of the total amount of the element. The method used should therefore be tested for the complexation efficiency of the complexants used, for each element. Several proven methods have been used in the certification. Natural ligands also interfere in

TABLE 8.24

SUMMARY OF THE TECHNIQUES OF FINAL DETERMINATION

Element	Techniques
Cd	APDCSV, DPASV, ETAAS, FPSA, IDMS, ZETAAS
Cu	APDCSV, DPASV, ETAAS, FPSA, IDMS, TXRF, ZETAAS
Mo	CSV, ICPMS, ZETAAS
Ni	ADPCSV, TXRF, ZETAAS
Pb	DPASV, ETAAS, FPSA, IDMS, TXRF, ZETAAS
Zn	DPASV, ETAAS, FPSA, IDMS, TXRF, ZETAAS

TABLE 8.25

CERTIFIED VALUES OF TRACE ELEMENTS IN SEAWATER CRM 403 IN nmol kg⁻¹

Elements	Certified values	Uncertainty	Number of accepted sets of results, p
Cd	0.175	0.018	15
Cu	3.90	0.37	13
Mo	103	20	3
Ni	4.36	0.36	10
Pb	0.117	0.025	9
Zn	25.7	2.9	9

TABLE 8.26

RESULTS OF THE EVALUATION OF CONSISTENCY OF THE METHODS USED FOR Cd, Cu AND Ni

Element	Tech. of final determination	CV% between means of same tech.	CV% between means of different tech.	Number of sets
Cd	DPASV	13.5	9.2	3
	ETAAS	19.5		6
	ZETAAS	15.1		3
Cu	ETAAS	13.5	0.5	3
	ZETAAS	5.9		4
Ni	ETAAS	10.0	11.5	3
	ZETAAS	11.2		4

electrochemical analysis. Some complexes will not be determined at the mercury electrode, or organic matter may adsorb to the electrode surface thus hampering electron transfer. In both cases, this will result in an underestimation of the total concentration. If complexation occurs during standard addition procedures, the concentration will be overestimated. To eliminate these interferences, photo-oxidation of the organic matter by UV-light is usually applied. Depending on the amount and nature of the organic matter, the irradiation of the acidified sample should last from 2–6 h; additional treatment with hydrogen peroxide is performed to enhance the breakdown of the organic matter [27].

8.8.6. Participating laboratories

The preparation of the seawater sample was performed by the IMW-TNO, Lab. Toegepast Marien Onderzoek in Den Helder (The Netherlands), whereas the homogeneity and stability studies were carried out at the Biologische Anstalt Helgoland in Hamburg

(Germany), the Labor für Spurenanalytik in Bonn (Germany), and the Rijkswaterstaat (Dienst Getijdewateren) in Den Haag (The Netherlands).

The following laboratories participated in the certification campaign: Biologische Anstalt Helgoland, Hamburg (Germany); Bundesamt für Seeschifffahrt und Hydrographie, Hamburg (Germany); Energieonderzoek Centrum Nederland, Petten (The Netherlands); IMW-TNO, Lab. Toegepast Marien Onderzoek, Delft (The Netherlands); Instituto Hidrográfico, Lisboa (Portugal); Instituut voor Nucleaire Wetenschappen, Rijksuniversiteit, Gent (Belgium); KEMA N.V., Arnhem (The Netherlands); Kemira Denmark A/S, Vedbaek (Denmark); Labor für Spurenanalytik, Bonn (Germany); MT-TNO, Analytische Scheikunde, Delft (The Netherlands); Nederlands Instituut voor Onderzoek der Zee, Den Burg (The Netherlands); Rijkswaterstaat, Dienst Getijdewateren, Haren (The Netherlands); Studiecentrum voor Kernenergie, Mol (Belgium); Universitaire Instelling Antwerpen, Wilrijk (Belgium); Università di Pisa, Dipartimento di Scienze, Pisa (Italy); University of Liverpool, Oceanography Laboratory, Liverpool (United Kingdom); Water Research Centre, Marlow (United Kingdom).

8.9. MERCURY IN SEAWATER

8.9.1. Introduction

Seawater and estuarine water CRMs certified for their contents of trace elements (see sections 8.7 and 8.8) were not analysed for mercury. Due to the high volatility of mercury, water to be analysed for Hg content can not be stored in polythene bottles and a higher acidification is necessary. So far, no CRM existed for this element, and hence a separate reference material had to be produced.

When selecting a site for the collection of the material, it was argued that most monitoring studies would be performed in coastal areas, and as a consequence a coastal seawater was preferred over an open ocean sample which, due to its lower Hg contents would be of use for only a limited number of users. A compromise was found in a sample with a content typically in the lower range of coastal waters. A feasibility study for the preparation of large batches of seawater for this element and results of inter-laboratory studies demonstrated that a certification campaign could be contemplated [28], and a candidate reference material was prepared for this purpose [29,30]. This material (CRM 579) was certified for its content of Hg.

8.9.2. Feasibility study

Previous exercises have shown the difficulties of determining Hg in seawater. As an example, coefficients of variation of 26.7 and 11.6% between eleven laboratories (CV of the mean of laboratory means) were found for Hg levels of respectively 6.0 and 24.2 ng L⁻¹ [31]. Therefore, it was chosen to consider seawater samples containing much higher Hg concentrations (coastal seawater samples spiked with mercury) in a first method performance study and to use natural coastal seawater, along with a spiked sample, in the second interlaboratory study.

In order to minimise contamination, all materials used in the exercises that came into contact with the seawater sample were made of either Linear Polyethylene (LPE) e.g. tanks, borosilicate glass (bottles) or teflon (PTFE or FEP) [28]. The cleaning procedure of the bottles, storage tank and tubing was based on earlier work [23]. In addition to the intensive cleaning procedures with acid solutions, all care was taken to minimize contamination during the various operations (cleaning, filtration, acidification, sample handling).

8.9.3. Interlaboratory studies

As mentioned before, two interlaboratory studies were organised prior to certification, involving ca. 15 laboratories using techniques such as cold vapour atomic absorption spectrometry, direct current plasma atomic emission spectrometry (DCP-AES), differential pulse anodic stripping voltammetry (DPASV), microwave plasma atomic emission spectrometry (MIP-AES), electrothermal atomic absorption spectrometry (ETAAS) and neutron activation analysis with radiochemical separation (RNAA).

In the first interlaboratory study, the examination of the raw data (14 sets of results of which 12 involved CVAAS, one RNAA and one MIP-AES) revealed a high spread of results due to two outliers. The mean obtained was $12.6 \mu\text{g L}^{-1}$ of Hg with a coefficient of variation (CV) between laboratories of 33%. The two high results were attributed to a laboratory contamination. The accepted values showed a picture which was found more acceptable, i.e. the mean obtained was $10.8 \mu\text{g L}^{-1}$ with a CV between laboratories of 6.6% [8]. At this stage, the agreement between the laboratories was found to be satisfactory; however, the Hg content in this (spiked) sample was considered much too high for being representative of natural samples which justified the organization of a second interlaboratory exercise for which results are described below.

In the second interlaboratory study, the main problems observed were due to contamination which explained high results both for the spiked and the natural sample. Additional sources of error were identified in the case of spiked sample analysis e.g. low results were explained both by a too long irradiation time in NAA (5 h instead of 2 h) which could have induced a volatilisation of Hg and an insufficient acidification possibly leading to the formation of SnO_2 (coprecipitation of Hg). It was mentioned that HCl for the acidification would be preferred over HNO_3 . In both cases (spiked and unspiked samples), the large scatter of data was partly explained by the small standard deviations (in some cases only 2%) which in any case allowed to detect systematic errors as shown by a Youden plot, plotting the results of the spiked versus non-spiked sample [28]. The CV between laboratories decreased from 140 to 10.3% in the case of the spiked sample results after removal of erroneous data. In the case of the natural sample, the CV decreased from 204 to 38% after removing erroneous data; this agreement was found to be too poor to allow certification and the state of the art of Hg in seawater was considered to be further improved if the preparation of a CRM is contemplated.

8.9.4. Preliminary work for the preparation of a candidate CRM

The preparation of a seawater candidate reference material to be certified for its content in mercury poses additional difficulties in comparison to other trace metals.

Firstly, mercury at low levels may display losses due to adsorption on the container wall if inadequate acidification is used and secondly, risks of contamination are likely either during the sample collection or the sample storage (e.g. from leaching of the container). The stability of mercury in aqueous solutions (distilled water) stored in various types of containers (polyethylene, polypropylene, quartz and glass) and acidified with different types of acids (HCl, HNO₃) showed that strong losses may occur due to adsorption and volatilisation e.g. for solutions acidified with HNO₃ and stored in polyethylene containers [32] whereas no detectable changes were observed for solutions treated with HCl/H₂O₂ and stored in polyethylene for up to two months; after this storage period, the losses were less than 2.5% for quartz, less than 5% for glass and less than 7% for polypropylene. These conclusions, however, concerned artificial solutions containing respectively 30 ng L⁻¹ and 1 µg L⁻¹ of Hg. In the preparation of the samples for the second intercomparison, it was considered that the surplus of the offered oxidation potential and the offered complexing reagent by the acidification with HNO₃ would be sufficient to maintain the stability of the Hg concentration in the seawater sample for years. Another argument for trusting a long-term stability of the samples was the difference in matrix effects; seawater contains high concentrations of cations and anions which adsorb in a larger extent than Hg cations. In the study of Krivan and Haas [32], only Hg cations were present in the solutions. In addition, the reaction sites were found to be lower in 1 L glass bottles (490 cm² L⁻¹) than in the vessels used in the mentioned study (3000 cm² L⁻¹). Finally, the experience has shown that the acidification of natural river and offshore waters with HNO₃ to a final concentration of 0.05 mol L⁻¹ allowed to preserve the stability of Hg for at least two years at ambient temperature [33].

On the basis of the results obtained in the preparation of the samples for the second interlaboratory study, it was concluded that the stability of a candidate seawater CRM for certification of its mercury content could be achieved for Hg following the procedure described in the section preparation. It was also considered that the Hg levels in a candidate CRM should be in the range of natural concentrations found in coastal seawater to make this material representative of analysis routinely performed. In the second interlaboratory study, the natural sample contained an average concentration of (4.1 ± 0.3) ng L⁻¹ of Hg as measured in the homogeneity and stability studies. A slight contamination by adsorbed mercury in the plastic surfaces of the tubes and tank was not excluded to explain apparently high mercury levels. A comparison with results found in the literature has shown, however, that such mercury contents are not uncommon in coastal and open ocean waters, e.g. Olafsson [34] found a mean concentration of (2 ± 1) ng L⁻¹ in north Atlantic waters and concentrations of around 3 ng L⁻¹ (with maximum levels of 10 ng L⁻¹) were found in the English channel waters [35]. A range of mercury concentrations of 1–5 ng L⁻¹ was therefore considered to be suitable for a candidate coastal seawater CRM.

8.9.5. Production of the candidate CRM

All materials used in the exercise that came into contact with the seawater sample were made of either Linear Polyethylene (LPE) (tanks), borosilicate glass (1 L bottles) or teflon (FEP) (for tubing) with the exception of a short length of silicone tubing in

the peristaltic pump head. General procedures followed guidelines previously published [28]. The materials were carefully cleaned according to procedures commonly used in trace metal analysis [23]:

- (1) Washing with a detergent to remove any grease, dust, particulate remnants and the like;
- (2) Washing with demineralised water;
- (3) Leaching with reagent grade nitric acid for 7 days, twice; in this and following cleaning procedures all bottles were completely filled;
- (4) Leaching with ultra pure nitric acid for 7 days;
- (5) Filling with ultra pure water acidified to $\text{pH} \approx 1.6$ with HNO_3 ; this solution was kept in the bottles/container until use.
- (6) Prior to the filling process, before opening of the bottles, the outside was washed with ultra pure water;
- (7) The acidified water was emptied from the bottle inside a clean bench;
- (8) The bottle was pre-washed with about 300 mL sample.

All operations (cleaning, filtration, acidification, sample handling) were carried out in a clean room atmosphere (Class 100) using clean benches. The bottling operation was carried out in a room specially adapted for the project. In order to minimise contamination, the ceiling, walls and floor were covered with heavy duty polythene foil. All operations with the seawater were carried out in a closed system, except for acidification and the filling of the bottles. These critical operations were carried out in a clean bench. All personnel working in the clean area used polythene gloves, dust-free garments and shoe-covers.

Seawater, available at the premises of the Royal Netherlands Navy's Chemical Laboratory, was sub-sampled. This coastal seawater was initially collected at about 10m water depth, in the nearby tidal channel (Marsdiep). The water passed a sand filter directly after collection, in order to remove larger particles and organisms. About 10m^3 seawater was thus collected in a glass-fibre tank and transported to the TNO Laboratory for Applied Marine Research, at Den Helder, where it was contained in a concrete basin, treated with a metal-free paint. In this basin the suspended particulate matter was allowed to settle for 4 weeks. This water served as source material for the candidate CRM, and had a salinity of 28 ‰.

Teflon tubing was connected to a peristaltic pump which in turn was connected to the on-line filter cartridge and then to the 2000 L storage container. Total length of the tubing was about 25 m. The first 50–60 L of the collected seawater were discarded. The seawater was collected directly in the tank. About 2000 L of seawater were thus sampled which took 7 h to complete.

The seawater was passed in-line over a $0.45\text{ }\mu\text{m}$ membrane filter cartridge placed after the peristaltic pump. After each filter change the first 20–30 L of seawater were discarded to allow for cleaning of the filter. Changing of filters was performed inside a clean bench. After collection the bulk seawater was acidified using ultra pure nitric acid to a pH of 1.5.

In order to achieve homogeneity in the tank, a metal free pump was used to circulate the seawater until bottling. This pump (30 L min^{-1} capacity) was set to circulate the water for 1 h in every 6 h. The filling of the (1 L) bottles was performed by using the

teflon tubing and peristaltic pump. After the cleaning solution was removed, the bottle was pre-washed with the sample solution. To enhance the between-bottle homogeneity, care was taken to perform the filling at a constant pump rate in one continuous operation. The bottles were filled with approximately one litre sample, leaving an air space to allow for future homogenisation. The bottling was performed inside a clean bench. The bottles were immediately closed with PPN caps. The bottles were packed in two self-seal bags and protected against breaking by polystyrene boxes. They were stored at ambient temperature in April 1995. About 1200 bottles, each containing ca. 1 L seawater, were prepared.

The between-bottle homogeneity was verified by the determination of total mercury by continuous flow cold vapour atomic fluorescence spectrometry (CVAFS) without pre-concentration. Prior to the analysis the samples were (high pressure) UV-irradiated to allow organic matter to be decomposed. Hg^{2+} was reduced by addition of SnCl_2 . The batch volume analysed was 30 mL, the volume of the gas-liquid separator was 10 mL. The CVs obtained (15.3% and 15.8%) were in the range of data that can be expected at this level of concentration. As a matter of comparison, CVs obtained by the certifying laboratories ranged between 2.5 and 12.8%, whereas two laboratories obtained CVs higher than 20%. The CV of the method used in the homogeneity study was 4.5% for a calibrant solution containing 5 ng kg^{-1} and was estimated to be in the range of 12–15% for a solution containing 2 ng kg^{-1} . In order to strengthen the conclusions of the above study, the homogeneity of the material was further studied on the basis of the results obtained by the certifying laboratories. The results showed that in some cases the within-bottle CV were in the same order of magnitude (ranging from 11.1 to 16.4%) that the CVs obtained in the homogeneity study; in general, however, the CVs were in the range 4–10%. It should be noted that these figures are more related to the method uncertainty than to a risk of within-bottle inhomogeneity. With respect to the between-bottle homogeneity, a 'nested design' evaluation showed that the between-bottle CV (based on certification measurements) is only 4.6%. On the basis of these data, it was hence concluded that the material homogeneity is suitable for use as a CRM and is representative of the present state of the art [29].

The stability of the total mercury contents was tested at $+20^\circ\text{C}$ over a period of 18 months. In addition, a short-term stability experiment was performed at $+40^\circ\text{C}$ to simulate worst-case transport conditions. No instability could be demonstrated and the material was considered suitable for certification. Transport at elevated temperature (40°C or below) for a short-term period (4 weeks or below) would not affect the stability of the material [29,30].

8.9.6. Certification

Two techniques were selected for certification, namely cold vapour atomic absorption spectrometry (CVAAS) and cold vapour atomic emission spectrometry (CVAES) applied after a variety of sample pretreatment, e.g. oxidation with BrCl or KmnO_4 , reduction with SnCl_2 , UV-irradiation, gold preconcentration etc.

High results obtained by RNAA raised questions regarding possible contamination. Verifications of the calibrant solution, chemical yield, flux corrections etc. proved that

these were not the cause for discrepancy. It was suspected that the sample could have been contaminated between filling of the plastic bags and the irradiation, i.e. the permeability of the polythene to Hg could have been the source for the problem. The RNAA set was therefore withdrawn from certification.

Two sets of CVAFS data were submitted by one laboratory of which one set (involving gold preconcentration) showed a high standard deviation (corresponding to a CV of ca. 23%); although these results were considered to reflect a realistic situation with respect to the state of the art, it was decided not to include them in the certification. The same was observed for a set of results obtained by ETAAS after complexation/extraction; while it was accepted that this technique has a higher variability (due to the larger number of handlings), the high standard deviation (resulting CV of ca. 26%) could not be accepted for certification. The two sets of results overlapped with the mean of laboratory means and were proposed as supporting values of $(2.64 \pm 0.61) \text{ ng kg}^{-1}$ and $(2.24 \pm 0.44) \text{ ng kg}^{-1}$.

From the various results of the homogeneity and stability studies, it was concluded that the part of the uncertainty that cannot be evaluated by the statistical analysis (e.g. related to matrix effects, method stability, blank variations etc.) was in the order of 10% (expressed as standard uncertainty). The uncertainty related to the certification exercise was equal to: $U_{\text{CERT}} = 0.9166/\sqrt{6} = 0.0374$ (or 2.0%), whereas the uncertainty factor related to homogeneity is 4.6% (see section 8.9.5). The combined uncertainty was thus estimated as follows: $U_c = [(2.0)^2 + (4.6)^2 + (10)^2]^{1/2} = 11\%$

A expanded uncertainty of 22% ($11\% \times 2$) was used for the certified value, using a coverage factor of 2. The certified value (unweighted mean of 6 accepted sets of results), and its calculated uncertainty, is equal to $(1.85 \pm 0.20) \text{ ng kg}^{-1}$ [29].

8.9.7. Participating laboratories

The collection, preparation, homogeneity and stability studies of the seawater samples for the interlaboratory studies were carried out by the TNO, Lab. Toegepast Marien Onderzoek in Den Helder (The Netherlands), the Biologische Anstalt Helgoland in Hamburg (Germany), the Labor für Spurenanalytik in Bonn (Germany), and the Rijkswaterstaat (Dienst Getijdewateren) in Den Haag (The Netherlands). The following laboratories participated in the interlaboratory studies: An Forais Taluntais, Dublin (Ireland); Aristotelian University, Thessaloniki (Greece); Danish Isotopecentralen, Copenhagen (Denmark); GKSS, Geesthacht (Germany); Instituto Hidrográfico, Lisbon (Portugal); IMW-TNO, Delft (The Netherlands); Bundesamt für Seeschifffahrt und Hydrographie, Hamburg (Germany); Universiteit Gent, Instituut voor Nucleaire Wetenschappen, Ghent (Belgium); Kernforschungsanlage, Jülich (Germany); Labor für Spurenanalytik, Bonn (Germany); Marine Pollution Laboratory, Thessaloniki (Greece); Rijkswaterstaat, Dienst Getijdewateren, Haren (The Netherlands); Energieonderzoek Centrum Nederland, Petten (The Netherlands); N.V. KEMA, Arnhem (The Netherlands); Presidio Multizonale di Prevenzione, Venezia (Italy); Presidio Multizonale di Prevenzione, Laboratorio Chimico, La Spezia (Italy); SCK, Mol (Belgium); Swedish Environmental Research Institute, Göteborg (Sweden); Universiteit

Antwerp, Dept. Scheikunde, Antwerp (Belgium); Università di Pisa, Dipartimento di Chimica, Pisa (Italy); Water Research Centre, Medmenham (United Kingdom).

8.10. Cr-SPECIES IN LYOPHILISED SOLUTION

8.10.1. Introduction

The different toxicity and bioavailability of Cr(III) and Cr(VI) are a public health concern and therefore require strict control. Trivalent chromium is found to be essential for man where it is involved in glucose, lipid and protein metabolism, whereas the deleterious effects to living organisms of Cr(VI) are well documented. Cr(VI) is also a potent carcinogenic agent for the respiratory tract requiring continuous monitoring of occupational air, e.g. risk assessment related to welding. Hence monitoring of the separate species in e.g. drinking water, occupational exposure or environmental samples is necessary. Determination of the total Cr content does not provide sufficient information about possible health hazards.

Standards were issued by the European Union (EU) for Cr in drinking water and occupational air. The maximum allowable concentration for chromium in drinking water is $50 \mu\text{g L}^{-1}$ according to the European Community Directive 80/778/EEC, L229/20, D48. Hexavalent chromium is such a potent carcinogenic agent for the respiratory tract that continuous monitoring is imposed, stated in the Directive 90/3941/EEC on exposure to carcinogenic substances. In occupational health, the OEL (Occupational Exposure Limits) for water soluble and certain water insoluble compounds in indoor air is limited to 0.5 mg m^{-3} for chromium, to 0.5 mg m^{-3} for Cr(III) and to 0.05 mg m^{-3} for Cr(VI) which reflects the different toxicity of both species.

The present state of the art of Cr speciation leaves much to be desired and compels to improve quality assessment. In order to meet the requirements of the directives, the reliability of the methods needs to be improved substantially. Appropriate reference materials certified for Cr(III) and Cr(VI), which are at present non existing, would provide both an effective and economical way for the laboratories to check and improve their performance which justified the production of a lyophilised solution for the certification of Cr-species [36,37].

8.10.2. Preliminary investigations

The stability of both Cr species was investigated in different media, different pH and different container materials. By choosing a hydrogen carbonate buffer solution at pH 6.4 as the agent to prevent hydrolysis of Cr(III), a matrix very close to that of real waters was achieved. Therefore initially an aqueous buffered sample, kept under a CO_2 blanket in sealed quartz ampoules, was foreseen. This evolved into 'lyophilised' samples to be reconstituted in the same buffer solution at the right pH. The optimal conditions for lyophilisation: avoiding losses of material, and the reduction of Cr(VI) to Cr(III), the optimal sample volume and measures to avoid possible adsorption on

the wall, etc were investigated, and kept under strict control. The work is described in more detail elsewhere [38].

8.10.3. Interlaboratory study

An intercomparison on European scale in which 24 laboratories participated was designed to identify pitfalls and sources of error and consequently to improve the skills of many laboratories to such a degree that certification of a reference material became feasible [39]. The intercomparison would also prove the suitability of the materials produced. Two hundred bottles of two different water samples were prepared according to the procedures developed by S. Dyg et al. [38]. The 'A' water sample was representative for tap or natural water, whereas the 'B' sample was intended to simulate a filter leaching solution in which much higher Cr(VI) concentrations were encountered. The B solution should help to detect analytical problems that are not connected with the leaching of the filters from monitors of stainless steel welders. The concentration range and stabilising matrix of both materials are indicated below:

Sample A: concentration range of Cr(III) and Cr(VI): 10–40 $\mu\text{g L}^{-1}$ in a $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer solution, pH 6.4, under a CO_2 blanket

Sample B: concentration of Cr(VI): 5–10 mg L^{-1} in a $\text{CO}_3^{2-}/\text{HCO}_3^-$ buffer solution, pH 9.6

A summary of the results obtained in the interlaboratory study is given in table 8.27.

For Cr(VI), high results found by some laboratories were attributed to various causes such as e.g. high blank value of the Amberlite LA-2 used for the separation of the Cr species, or inadequate separation. Low values found by other laboratories were suspected to be due to incomplete extraction which was supported by the fact that their Cr(III) value was too high.

The overall picture revealed that most laboratories had difficulties in determining the Cr(III) content of the A solution. The reason was mainly related to too high detection limits. High values were suspected to be due to contamination problems whereas low

TABLE 8.27

RESULTS OF THE INTERCOMPARISON FOR Cr SPECIATION IN A LYOPHILISED SOLUTION

Cr species	Mean of means and S.D.	Initial content made up
Cr(VI) in A solution	$14.07 \pm 1.12 \mu\text{g L}^{-1}$	$14.11 \mu\text{g L}^{-1}$
Cr(III) in A solution	$22.24 \pm 3.01 \mu\text{g L}^{-1}$	$25.14 \mu\text{g L}^{-1}$
total Cr in A solution	$39.55 \pm 2.88 \mu\text{g L}^{-1}$	$39.25 \mu\text{g L}^{-1}$
Cr(VI) in B solution	$5.64 \pm 0.40 \text{mg L}^{-1}$	5.644mg L^{-1}
total Cr in B solution	$5.62 \pm 0.26 \text{mg L}^{-1}$	5.644mg L^{-1}
Cr(VI) in filters	$34.5 \pm 1.5 \text{mg per kg dust}$	

values could be explained by incomplete extraction of Cr(VI) with Amberlite as already mentioned above. One set of results was determined by ETAAS after its complexation with quinolin-8-ol and extraction into MIBK; the laboratory experienced problems with sputtering of the sample due to evaporation of CO₂ from the carbonate buffer when heating the sample for the complexation of Cr(III), which explained the rather low results.

The determination of the total Cr content in the A solution did not cause many problems.

The outcome of this intercomparison was promising enough to justify a certification exercise on a material identical to the A solution with slightly different concentrations.

8.10.4. Production of the candidate CRM

A batch of 1100 vials containing a lyophilised solution, similar to the previous 'A sample' was prepared. The preparation of the samples was performed under class 100 clean room facilities. All laboratory ware: pipettes, volumetric flasks etc. was thoroughly cleaned by leaching in diluted acids.

1.009 g Na₂CrO₄·4H₂O and 0.7067 g CrCl₃·6H₂O (analytical grade) were weighed and dissolved in 100 mL Milli-Q water or 100 mL HCl (subboiled, 0.1 mol L⁻¹) respectively for the initial stock solutions. They were diluted 10 times, and 5–10 mL of the Cr(VI)/Cr(III) stock solution respectively were transferred to a 5 L volumetric flask. 21 g NaHCO₃ (p.a. grade) and 124 mL HCl (subboiled, 1 mol L⁻¹) were then added to obtain the HCO₃⁻/H₂CO₃ buffer solution, pH 6.4. The whole was made up to volume with Milli-Q water and kept under a CO₂ blanket. 2 mL of the solution was pipetted into cleaned brown glass vials and weighed. All individual weights are given in the certification report [36]. The concentration of Cr(VI) and Cr(III) is calculated to be 22.42 µg L⁻¹ and 27.58 µg L⁻¹ respectively, after reconstitution in 20 mL of buffer. The samples were deep frozen for at least 12 h prior to lyophilisation. The lyophilisation was done under carefully controlled conditions to avoid losses of material due to sputtering, reduction of Cr(VI), or adsorption on the wall. Due to the limited volume of the freeze drying equipment, the lyophilisation had to be done in 7 batches. The homogeneity test did not reveal any systematic differences between the different batches.

When the freeze drying was completed, the equipment was purged with pure N₂ and the bottles were sealed with Butyl stoppers with a teflon coating, and an alumina cap. Purging with N₂ ensures the samples to be stored under an inert atmosphere thus preventing possible reduction of Cr(VI). The samples were further stored at 5°C.

To check the between bottle homogeneity, 50 vials were randomly set aside after lyophilisation. They were taken from the seven lyophilisation batches. The lyophilised samples were reconstituted with 20 mL of HCO₃⁻/H₂CO₃ buffer, pH 6.4. The separation of Cr species took place immediately after reconstitution of the sample. The separation method is based on the cationic behaviour of Cr(III) and the anionic behaviour of Cr(VI). It relies on an ion exchange extraction using the liquid anion exchanger Amberlite LA-2. The liquid anion exchange solution (LAES) is obtained by stripping the Amberlite in HCl (6 mol L⁻¹) and diluting it in methyl isobutyl ketone (MIBK). Cr(VI) is extracted

completely into the organic phase while Cr(III) remains in the aqueous phase. 2 mL of the reconstituted samples and 1 mL of LAES were mixed on a whirling mixer for 1 min and centrifuged at 2500 rpm for 10 min. The two phases were separated and analysed for their Cr content by ETAAS (Perkin Elmer 4100 ZL). Cr(VI) was measured in the organic phase and Cr(III), after 2 fold dilution, in the aqueous phase. For the determination of the total Cr content the reconstituted sample was diluted 4 times. The samples were measured against matrix matched calibration curves. No systematic difference at the 0.05 significance level was observed between the CV of the results from the 50 bottles and the CV of the method for Cr(VI) and total Cr. For Cr(III) no difference could be observed at the 0.01 significance level. It could therefore be concluded that the samples are homogeneously distributed over the different vials [36].

The stability of the contents of the Cr(VI) and Cr(III) species was tested at +5°C and +20°C respectively, over a period of 12 months. The samples were analysed using the same procedures as for the homogeneity study. No instability could be demonstrated [36].

8.10.5. Certification

The methods used by the participants are summarised in table 8.28. In the case of ETAAS detection, extraction was generally performed with liquid anion exchange solution (Amberlite LA 2/MIBK).

Some of the laboratories reconstituted the lyophilised samples in H₂O instead of the HCO₃⁻/H₂CO₃ buffer solution. This does not influence the results as such, but enhances the risk for reduction of Cr(VI). Reconstitution in HCO₃⁻/H₂CO₃ buffer is therefore recommended.

One laboratory reported results obtained after storing the reconstituted samples in the dark under a CO₂ blanket for a few weeks. The Cr(VI) results were much lower while the Cr(III) results were much higher than the ones obtained directly after reconstitution and they did not agree with the rest of the laboratories. Clearly a reduction of Cr(VI) has occurred in the reconstituted sample during storage. It is therefore recommended to start the separation of the species as soon as possible after reconstitution.

The certified values, along with their uncertainties, are given in Table 8.29.

TABLE 8.28

SUMMARY OF TECHNIQUES USED

Elements	Technique
Cr(III)	ETAAS; micro-column/ICPMS; IDMS
Cr(VI)	DPCSV; ETAAS; IC/SPEC with DPC; IC/CHEMI; Micro-column/ ICPMS; IDMS
Total Cr	DPCSV; ETAAS; ICPAES; IDMS; INAA

TABLE 8.29

CERTIFIED VALUES FOR Cr(III), Cr(VI) AND TOTAL Cr IN BCR CRM 544,
EXPRESSED IN mg L^{-1}

Component	Certified value	Uncertainty	p
Cr(VI)	22.8	1.0	13
Cr(III)	26.8	1.0	9
total Cr	49.4	0.9	14

8.10.6. Participating laboratories

This project has been coordinated by the Universiteit Gent, Instituut voor Nucleaire Wetenschappen (Belgium) which also prepared the candidate CRMs and verified their homogeneity and stability. The following laboratories participated in the certification campaign: Arbejdsmiljøinstituttet, Dept. of Chemistry and Biochemistry, Copenhagen (Denmark); Bayer Antwerpen, Centraal Analytisch Labo, Antwerpen (Belgium); Berufsgenossenschaftliches Institut für Arbeitssicherheit, Sankt Augustin (Germany); Ciba-Geigy Ltd., Central Analytical Dept., Basle (Switzerland); Fond. Clinica del Lavoro, Lab. di Igiene Industriale, Pavia (Italy); General Chemical State Laboratory, Athens (Greece); Health and Safety Executive, Sheffield (United Kingdom); Institut für Anorganische Chemie, Univ. Regensburg (Germany); Institut Pasteur, Service Eaux et Environnement, Lille (France); Institute of Occupational Health, Helsinki (Finland); Institute of Occupational Health, Oulu (Finland); Laboratorium voor Analytische Scheikunde, Universiteit Gent (Belgium); Oceanography Laboratory, University of Liverpool (United Kingdom); School of Science, Sheffield Hallam University (United Kingdom); University of Plymouth, Dept. Environm. Sciences, Plymouth (United Kingdom); Vandkvalitetsinstituttet, Hørsholm (Denmark); VITO, Mol (Belgium).

8.11. Se-SPECIES IN SOLUTION

8.11.1. Introduction

There is a growing interest in the determination of chemical forms of selenium in environmental matrices, owing to the different biological and toxic effects of the various species. Environmental studies dealing with selenium have mostly focused on the determination of inorganic species (selenite and selenate) and organic compounds such as dimethylselenium and dimethyldiselenium (released into the air from soils, lake sediments and sewage sludge), selenocysteine and selenomethionine, and trimethylselenium. The determination of the different forms of selenium in the environment is necessary owing to the different effects related to their respective chemical forms: species can either be considered as essential, e.g. selenite is added to foodstuffs to care for selenium deficiency problems, or highly toxic, e.g. selenate compounds.

Techniques developed for the determination of selenite and selenate involve a succession of several analytical steps (e.g. reduction, separation, detection) which are often far from being validated. In addition, the knowledge related to the stability of the species is still very scarce. A project has hence been launched within the BCR programme with the aim to evaluate the stability of Se-species in solution [42]; this feasibility study has been continued by an interlaboratory study for the evaluation of method performance [43]. Both investigations were designed to improve the state-of-the-art of Se-speciation prior to the tentative certification of solution candidate reference materials as described in this section. As a follow-up, artificial freshwater solutions containing inorganic Se-species were prepared (RMs 602 and 603) [40,41].

8.11.2. Preliminary investigations

Many problems occur in Se-speciation analysis, owing e.g. to risks of adsorption on container walls, instability of species or contamination, insufficient separation efficiency of the chromatographic techniques, problems of conversion yield of selenite to selenate etc. Prior to conducting an interlaboratory project on this topic, it was hence decided to assess the stability of selenite and selenate according to various factors (effects of container materials, additives, temperature and light). The study focused on tests of effects of physicochemical parameters on solutions stored in polyethylene and PTFE containers. Container volumes were 100 and 500 mL for polyethylene and 500 and 1000 mL for PTFE. Stock and initial working solutions were prepared in 1 and 5 L polyethylene containers previously cleaned with nitric acid (at pH 2) and rinsed with Milli-Q water. The stock solutions were prepared with sodium selenite and sodium selenate (purity >98%).

Eight initial working solutions with a total volume of 10 L were prepared. Two solutions with concentrations of 10 and 50 $\mu\text{g L}^{-1}$ of each species, each of them at two different pH values (pH 2 by adding H_2SO_4 and pH 6 with no addition of extra reagent), with and without addition of chloride (100 mg L^{-1} as NaCl), were prepared in polyethylene containers. The homogeneity of the solutions was achieved by continuous pumping with a peristaltic pump and PTFE tubes for 5 h. Oxygen was removed from bottles by bubbling with N_2 . These initial working solutions were placed in 100–500 mL polyethylene and 500–1000 mL PTFE containers kept in the dark at -20 , 20 and 40°C and exposed to sunlight at 20°C . Solutions were stored and total selenium and selenite were determined after 1 day, 2 weeks, and 1, 2, 6, 9 and 12 months. Determinations of total Se were carried out by hydride generation atomic absorption spectrometry after prereduction of selenite to selenate with $\text{HCl } 6 \text{ mol L}^{-1}$; independent measurements were performed by neutron activation analysis for quality control. The solutions were found to be stable at -20°C in all conditions tested (pH 2 and 6, with and without chloride). At ambient temperature, samples stored at pH 2 in polyethylene containers showed instability of selenite after one month storage whereas selenate remained stable; this difference in behaviour was attributed to possible adsorption of selenate onto the container walls. The stability was better at pH 6 but instability of selenite was still observed after two months storage, whereas selenate remained stable. When a PTFE

container was used, dramatic losses of selenite at low concentration level were observed at pH 6.

The effect of storage at 40°C was studied in 100 mL vessels (instead of 500 mL as used in the other experiments). Surprisingly, the stability was found to be much better for both species in solutions stored at pH 2 and pH 6 in polyethylene containers (with and without addition of chloride). Tests performed with samples stored in the dark and exposed to sunlight demonstrated that light had no significant effect on the stability of selenite and selenate for the period tested.

At this stage, the conclusions of the feasibility study were that the optimum temperature at which there is no risk of selenium losses at the 10 and 50 $\mu\text{g L}^{-1}$ levels over 12 months is -20°C . The stability of the species at both 20 and 40°C depends upon the pH and the container type. Generally, both selenite and selenate stored in polyethylene containers at 40°C appeared to be more stable than at room temperature, particularly at pH 6. The presence of chloride tended to stabilize both species.

On the basis of these preliminary conclusions, further experiments were carried out by spiking the solutions with increasing concentrations of Cl^{-} (up to 20000 mg L^{-1} , simulating the salinity of seawater); this additional study was performed by preparing a series of selenite/selenate solutions containing approximately 10 and 50 $\mu\text{g L}^{-1}$ of each species at pH 6 and spiking them with Cl^{-} concentrations of 100, 50, 1000, 2000, 3000 and 5000 mg L^{-1} ; a set of solution was also prepared with seawater (corresponding to 20000 mg L^{-1} of chloride). Samples were maintained in polyethylene containers in the dark at ambient temperature for one year. The suitability of HCl addition at pH 2 for stabilizing Se-species in comparison to H_2SO_4 was also tested; the solutions were prepared with a single addition of HCl ($\text{Cl} = 350 \text{ mg L}^{-1}$) and stored in the dark in polyethylene containers at ambient temperature for one year. The results obtained in these new storage conditions showed that selenate was stable for one year in all conditions tested while selenite was unstable in 0.01 mol L^{-1} HCl after two months storage; these results agreed with those obtained with H_2SO_4 in the feasibility study. The selenite stability improved with the addition of increasing Cl^{-} concentrations and this species was completely stabilized for 12 months as tested in seawater. It should be noted that the prepared samples were opened every two months (6 times) for analysis; however, previous studies have shown that the stability of these species decreases when the head volume of the container increases. In order to check whether the stability of selenite would be improved in solutions containing 2000 mg L^{-1} of Cl^{-} stored in tightly closed containers, solutions containing 6 $\mu\text{g L}^{-1}$ of selenite, 6 $\mu\text{g L}^{-1}$ of selenate and 2000 mg L^{-1} of Cl^{-} were stored at room temperature and analyses were performed every week. Both Se-species were found to be stable over 18 months when the containers were completely full and opened only once for analysis. However, when the same bottle was analysed at different times, selenite was only stable for two weeks (bottles being opened 5 times) whereas selenate was stable in all conditions tested.

The conclusions of the overall study were that a Cl^{-} concentration of 2000 mg L^{-1} or more is suitable to stabilize selenite but samples have to be opened only at the time of analysis to ensure a complete stability. This recommendation was clearly stressed to participants in the first interlaboratory study (see below).

8.11.3. Interlaboratory study

Results of selenite determination: Sources of error detected in the technical discussion were mainly due to calibration errors or lack of quality control. It was stressed by the participants using ICPMS that polyatomic interferences from Cl^- can be removed to improve the accuracy of selenite determination; ways to do so are (i) to dilute the original sample after pre-concentration of selenite, (ii) to modify the plasma conditions by adding N_2 carrier to the plasma or (iii) to use anion-exchange chromatography to separate chloride from the selenium species. When diluting and pre-concentrating on a precolumn, care is to be taken that selenite is quantitatively recovered. The use of silver nitrate to remove chloride is suspected to affect selenite. The coefficient of variation (CV) between laboratories (raw data) was 23.7% for solution A; it decreased to 6.2% after removing outliers on technical grounds. The mean of laboratory means was $(5.6 \pm 0.4) \mu\text{g L}^{-1}$ which overlapped the expected value of $6.0 \mu\text{g L}^{-1}$. The CV between laboratories (raw data) was 17.5% for solution B; it decreased to 6.3% after removing outliers on technical grounds. The mean of laboratory means was $(51.0 \pm 3.2) \mu\text{g L}^{-1}$ which matched well with the expected value of $50.0 \mu\text{g L}^{-1}$.

Results of selenate determination: The discussion focused on how to efficiently reduce selenate to selenite prior to hydride generation and how to calculate the recovery. Since selenite calibrant was used to verify the efficiency of the reduction process, the laboratories were recommended to apply the same analytical procedure to both samples and calibrants. It was stressed that the HCl molarity and the reduction temperature should be strictly controlled. HCl molarities ranging between 4 and 6 mol L^{-1} and temperatures ranging between 60 and 100°C (with different heating times) were found to be suitable to ensure a complete reduction of selenate to selenite. The determination of selenate by difference between total selenium content and selenite was questioned. Although this method is quite commonly used and acceptable for selenate determination, this does not correspond to a direct measurement of the species; hence, this method would not be accepted for certification and it was proposed that the results obtained by difference would be used as confirmative values of results obtained by techniques actually separating the Se-species (e.g. HPLC-ICPMS or HPLC-HGAAS). For solution A, the CV between laboratories (raw data) was 31.9%; it decreased to 4.1% after removing outliers on technical grounds. The mean of laboratory means was $(6.2 \pm 0.3) \mu\text{g L}^{-1}$ which overlapped the expected value of $6.0 \mu\text{g L}^{-1}$. The CV between laboratories (raw data) was 29.4% for solution B and decreased to 7.0% after removing outliers on technical grounds. The mean of laboratory means was $(50.3 \pm 3.6) \mu\text{g L}^{-1}$ which overlapped the expected value of $50.0 \mu\text{g L}^{-1}$.

8.11.4. Production of the candidate CRMs

Two 250 L polyethylene tanks were used for the preparation and homogenisation of the two candidate CRMs; they were cleaned thoroughly with deionized water (Milli-Q) and subsequently rinsed with the solution they would contain. Polyethylene bottles were carefully cleaned with deionized water (Milli-Q); each bottle was rinsed with the solution they would contain prior to final bottling. The bottle caps were treated in a

similar way. The two candidate CRMs were prepared from deionized water (Milli-Q) to which the compounds of interest were added in the form of sodium salts (Na_2SeO_3 and Na_2SeO_4); the chloride added to stabilize selenium species were spiked as NaCl .

The target values obtained (gravimetrically) were:

RM 602	RM 603
$(6.0 \pm 0.1) \mu\text{g L}^{-1}$ selenite	$(35.0 \pm 0.8) \mu\text{g L}^{-1}$ selenite
$(8.0 \pm 0.2) \mu\text{g L}^{-1}$ selenate	$(45.0 \pm 1.0) \mu\text{g L}^{-1}$ selenate

Homogenisation was carried out in the polyethylene tanks covered with a close fitting polyethylene lid. A centrifugal pump connected to the tank with polyethylene piping ensured constant recirculation of the solution. The pump had no metallic parts in contact with the water. The bottling was performed manually using pre-rinsed polymer tubes, avoiding any contact with metals. Each bottle was sealed and stored at ambient temperature. The between-bottle homogeneity was verified by HPLC-HG-AAS. A preconcentration step was necessary in the case of the solution with low content (RM 602); this was achieved by retaining the selenium species on an alumina microcolumn conditioned in anionic form with nitric acid, and eluting selenite and selenate with 2 mol L^{-1} ammonia solution. No inhomogeneities of the material were suspected [40].

The stability of the selenite and selenate contents was tested at $+20^\circ\text{C}$ over a period of 12 months and both compounds were determined at regular intervals during the storage period using the same procedures as for the homogeneity study. No instability could be demonstrated over 12 months [40]. Problems of leakage were observed in some of the bottles containing the reference material, which justified an additional stability study. Consequently, it was decided to test other polypropylene bottles with tighter caps for the storage of the reference materials. This additional study was carried out 24 months after the initial stability study and led to the detection of instability problems of the Se-species over a long-term period. A clear decrease in selenite content was observed in the new polypropylene bottles after 8 months storage which was particularly acute for the low-concentration reference materials whereas selenate remained stable over the same period. On the basis of these results, it was found necessary to control the stability of the Se-species in the materials stored in the original polypropylene bottles as well as in the stock solution stored in the polyethylene tank; the results showed that both species were stable for 36 months at the two concentration levels in the polyethylene (60 L) tanks whereas a decrease of around 30% and 15% was observed for selenite in the two solutions stored in polypropylene bottles over the same period; selenate was found to slightly increase over the same duration but this change is not significant. The reasons for this instability could be attributed to an adsorption process onto the container surface which was not observed in the 60 L tank, owing to a much smaller surface/volume ratio; in other terms, the ratio is 11 times smaller in the storage tanks in comparison to the 100 mL bottles, leading to a better stability of the species. Furthermore, the polyethylene material of the tank seems to be more suitable to achieve stability in comparison to polypropylene. On the basis of these additional studies, it was impossible to propose the materials for certification.

8.11.5. Tentative certification

The techniques used in the certification are summarized in Table 8.30.

In the case of selenite, calibration or calculation errors justified the rejection of 4 laboratory sets of results on the 23 sets submitted. Problems were experienced with ICPMS, owing to the matrix effects of Cl⁻; while three of the four laboratories using ICPMS could correct these effects, one set was rejected due to high (outlying) results. Oxidation of selenite to selenate was suspected in two cases, in particular with the CSV technique, explaining low results that were withdrawn. Finally, high standard deviations were obtained with CCG-MIPAES and GC-FPD techniques; this was considered to be inherent to the GC technique and the results were, consequently, accepted. No particular problems were noticed for total Se in the two materials.

In the case of selenate, as stressed in the interlaboratory study, the results obtained

TABLE 8.30

TECHNIQUES FOR Se-SPECIATION

a. Summary of techniques used for selenite determination

Diethylselenide formation; trapping in fused silica column; capillary GC; MIP-AES detection (Lab.01)

Hydride generation (1.5% NaBH₄); addition of HCl; AFS detection (Lab.02)

Hydride generation (0.2% NaBH₄); addition of HCl; AFS detection (Lab.03)

Hydride generation (0.5% NaBH₄); addition of HCl; alumina preconcentration; AAS detection (Lab.04)

Hydride generation (1.5% NaBH₄); addition of HCL; AFS detection (Lab.06)

Hydride generation (0.2% NaBH₄); addition of HCl; ICP-MS detection (Lab.07)

Hydride generation (2% NaBH₄); addition of HCl; AAS detection (Lab.10)

Hydride generation (0.2% NaBH₄); addition of HCl; QFAAS detection (Lab.11)

HPLC with anion exchange; ICP-MS detection (Lab.11)

Hydride generation (0.2% NaBH₄); addition of HCl; ICP-MS detection (Lab.11)

HPLC with anion exchange; ICP-MS detection (Lab.12)

Hydride generation (0.5% NaBH₄); addition of HCl; AAS detection (Lab.13)

Hydride generation (1.5% NaBH₄); addition of HCl; AFS detection (Lab.14)

Alumina preconcentration; elution with HNO₃; ICP-MS detection (Lab.15)

On-line preconcentration by chelation and C₁₈ microcolumn; elution with ethanol; ETAAS detection (Lab.16)

B: SUMMARY OF TECHNIQUES USED FOR SELENATE DETERMINATION

Hydride generation; alumina preconcentration; HPLC; on-line microwave reduction of selenate to selenite in HCl; total determination as selenite; detection by AAS (Lab.04)

HPLC with anion exchange; ICP-MS detection (Lab.11)

HPLC with anion exchange; ICP-MS detection (Lab.12)

by difference between total selenium content and selenite were not accepted for certification. Indeed, although this method is quite commonly used and acceptable for selenate determination, it does not correspond to a direct measurement of the species. The certification of selenate was hence based on the results of techniques actually separating the Se-species (e.g. HPLC-ICPMS or HPLC-HGAAS), with a gravimetric confirmation (target values). The values were also confirmed by alternative techniques determining selenate by difference; the mean of laboratory means were, respectively, $(7.7 \pm 0.7) \mu\text{g L}^{-1}$ and $(44.8 \pm 4.4) \mu\text{g L}^{-1}$.

The mean of the selected values (originally proposed for certification) and their uncertainties (half width of the 95% confidence intervals) are given in the tables 8.31a and 8.31b. As mentioned above, the values of selenate were confirmed by determinations based on the difference between total Se and selenite.

As stressed in the section 8.11.4, the instability observed (after the certification campaign) did not enable to accept the materials as CRMs. The results of this tentative certification show that (1) the state-of-the-art for Se-speciation is good enough for laboratories to obtain comparable data, (2) reference materials containing inorganic Se-species can be stabilised and used over a 12-month period for the purpose of interlaboratory studies or routine quality control checks, (3) work remains to be done to find the optimal storage conditions for candidate CRMs to enable long-term stability and availability of the materials. An important aspect of this work is that reference materials can be prepared by laboratories for their own quality control, following the 'cooking recipe' given in this section; it is obvious that such reference solutions should be used with all necessary care to avoid instability problems and should certainly not be kept over a period longer than 12 months.

8.11.6. Participating laboratories

The overall coordination and preparation of the CRMs has been conducted by the Universidad Complutense, Departamento de Química Analítica in Madrid (Spain). The following laboratories participated in the certification campaign: CISE, Milano (Italy); CNRS, Service Central d'Analyse, Vernaison (France); CSIC, Departamento de Química Ambiental, Barcelona (Spain); De Montfort University, Department of

TABLE 8.31A

ASSIGNED VALUES OF TOTAL SELENIUM, SELENITE AND SELENATE IN RM 602

Component	Assigned value and uncertainty	Target value (obtained gravimetrically)	P
Total Se	13.5 ± 0.4		18
Selenite	5.9 ± 0.2	6.0 ± 0.1	14
Selenate	8.1 ± 0.3	8.0 ± 0.2	3

TABLE 8.31B

ASSIGNED VALUES OF TOTAL SELENIUM, SELENITE AND SELENATE IN RM 602

Compound	Assigned value and uncertainty	Target value (obtained gravimetrically)	P
Total Se	80.3 ± 1.4		16
Selenite	34.9 ± 0.8	35.0 ± 0.8	14
Selenate	45.1 ± 2.4	45.0 ± 1.0	3

Chemistry, Leicester (United Kingdom); Institut Pasteur, Service Eaux et Environnement, Lille (France); EHICS, Laboratoire de Chimie Minérale et Analytique, Strasbourg (France); National Food Agency, Søborg (Denmark); Perkin-Elmer, Überlingen (Germany); School of Analytical Sciences, Dublin (Ireland); Technische Universität Wien, Institut für Analytische Chemie, Wien (Austria); Universidad Complutense, Departamento de Química Analítica, Madrid (Spain); Universidad de Córdoba, Departamento de Química Analítica, Córdoba (Spain); Università la Sapienza, Dipartimento di Chimica, Roma (Italy); Université de Bordeaux, Laboratoire de Photophysique et Photochimie Moléculaire, Talence (France); Université de Pau, Laboratoire de Chimie Analytique, Pau (France); Universiteit Antwerp, Dept. Scheikunde, Antwerp (Belgium); University of Plymouth, Department of Environmental Sciences, Plymouth (United Kingdom); University of Sheffield, Centre for Analytical Sciences, Sheffield (United Kingdom); University of Southampton, Department of Chemistry, Southampton (United Kingdom).

8.12. UNSTABLE PESTICIDES IN LYOPHILISED SOLUTION

8.12.1. Introduction

Pesticide formulations are widely employed in modern agriculture for the protection of the crops. Some of them disappear rapidly but others remain for longer periods in soils, groundwaters and rivers. Within the European Community, millions of tons of pesticides are used every year in agriculture, which led to surface water pollution and consequently drinking water. The determination of many of these pesticides still poses difficult problems, especially with regards to accuracy due to the lack of stability of these compounds in aqueous solutions. One of the major problems to monitoring the current generation of pesticides is the unavailability of certified reference materials to evaluate the performance of the analytical systems [44]. As a response to this need, a freeze-dried water reference material was prepared to improve the state of the art of polar pesticide determinations in water, which offered a mean to verify the quality of these determinations through the production of a CRM. Seven compounds were certified (CRM 606), namely: atrazine, simazine, carbaryl, linuron, propanil, permethrin, fenamiphos [45].

8.12.2. Production of the reference material

A batch of 6000 L of tap water was spiked with NaCl (2.5 g L^{-1}) and with atrazine, simazine, carbaryl, propanil, linuron, fenamiphos and permethrin. The final concentration of each pesticide was $50\text{--}80 \mu\text{g L}^{-1}$ ($200\text{--}320 \mu\text{g L}^{-1}$ of permethrin). A blank of the same water (3000 L) also containing 2.5 g L^{-1} NaCl was prepared. The solution was freeze-dried to -15°C on the trays and then started the refrigeration of the condenser. Once a temperature of -40°C is reached, the vacuum was applied till the pressure is $100 \mu\text{m}$, and then starts the heating of the system up to 50°C . In all cycles the temperature was 50°C . The vacuum range was between 40 to $450 \mu\text{m}$.

Once the lyophilization was completed, the residue was separated from the trays, weighed and placed into aluminium containers. The residue was weighted in each cycle, obtaining a total amount of 7.2 kg and 14.3 kg of blank and sample residue respectively. An average yield of 80% was obtained. The contents of the aluminium containers with pesticides-enriched material were put into a polyethylene container and homogenised in a turbo mixer for 30 minutes. The material was sieved ($> 0.250 \text{ mm}$) to eliminate salt lumps. Finally, the sieved material was homogenised in a turbo mixer for two hours.

Both materials, without pesticides (blank) and with pesticides (spiked samples), were bottled in amber glass vials using a fully automatic filling machine for powders. About 2.8 g of salt powder was introduced into each amber vials whose were closed and filling with 99.995% argon with teflon faced rubber lids, held in place with aluminium caps. The CRM 606 consists of a set of three samples: one blank and two spiked pesticides, packed in a polyethylene bag. Additionally 160 vials of spiked salt were available for the homogeneity, stability and certification exercise.

500 mg of the residue were transferred to a clean glass bottle and were dissolved in 0.5 L of $10^{-3} \text{ mol L}^{-1}$ HCl at ambient temperature ($20\text{--}25^\circ\text{C}$) [46]. Direct light illumination is to be avoided during all operations to avoid photolysis of the compounds studied. A clean Teflon stirrer bar was inserted into the solution. The pesticide determination analysis was started immediately after reconstitution. After reconstitution of 500 mg of freeze-dried material with 0.5 L of $10^{-3} \text{ mol L}^{-1}$ HCl, 40 mL were used for an on-line LC analysis. A chromatographic precolumn ($10 \text{ mm} \times 4.6 \text{ mm i.d.}$) packed with $5 \mu\text{m}$ ODS was coupled with the loop of a Rheodyne 7725i injection valve. The solid-phase extraction precolumn was first conditioned with 10 mL methanol and then 10 mL Milli-Q water at 1 mL min^{-1} using a high pressure pump. Water samples were preconcentrated at a flow-rate of 4 mL min^{-1} . Following the preconcentration step, the injection valve was switched and the analytes were eluted in a back-flush mode and separated in a chromatographic column ($25 \text{ cm} \times 4.6 \text{ mm i.d.}$) packed with $5 \mu\text{m}$ ODS. A gradient elution with a flow-rate of 1 mL min^{-1} was performed as follows: the pesticides were detected with a diode-array detector at 220 nm for atrazine, simazine and carbaryl and 250 nm for propanil, linuron and fenamiphos. Quantitation was performed by linear calibration using peak area measurements and fenitrothion as internal standard. The results (CV) of the within- and between-bottle homogeneity obtained for simazine, carbaryl, atrazine, propanil, linuron and fenamiphos did not demonstrate any within- or between-bottle inhomogeneity for a sample intake of 500 mg . However the distribution of permethrin in the samples was inhomogeneous (Mean value: $113.19 \text{ mg kg}^{-1}$; C.V.

(%): 125.59 ($n=5$) in one bottle) which made the CRM not suitable for further studies of this pesticide [45].

The method used to carry out the stability study was the same to the one used for the homogeneity study. At -20°C over a storage period of 12 months, pesticides contents in the material did not show any losses, so it was concluded that no instability could be demonstrated. At $+4^{\circ}\text{C}$ all pesticides seem to be stable for at least 9 months, except fenamiphos. At $+20^{\circ}\text{C}$ all pesticides were stable during the first month of storage and slight losses of different pesticides (simazine, carbaryl, atrazine, fenamiphos) were detected. Based on the results of the stability study it was recommended to store the samples at -20°C in the dark. In order to evaluate the stability of the material during the transport, the candidate CRM was tested at $+40^{\circ}\text{C}$ at short time intervals (each 2 days). Results obtained detected significant losses of all pesticides in the material after two days of storage. Thus it was concluded that mailing should not last longer than 2 days if any risk of having temperature higher than $30\text{--}40^{\circ}\text{C}$ would exist [45].

8.12.3. Certification

All the selected laboratories for the certification exercise had already been involved in an earlier interlaboratory exercise in order to validate the analytical methods used [44]. An estimation of the recovery of the pesticides from one liter of water was requested by adding a known amount of each pesticide or by reextraction. For chromatographic analysis at least the use of one internal standard was necessary. The reconstitution process had to be strictly followed by all laboratories.

Each laboratory prepared separate calibrants according with their own laboratory procedure. These were used for calibrating the detector within its dynamic range. Calibrants were prepared, avoiding serial dilution. A minimum of five calibration points were required. The coordinator supplied the participants with pure pesticides samples with certified purity. The participants were requested to use these calibrants or to check their own calibrants. For GC and HPLC analysis at least one internal standard was used for the final determination.

Sample dissolution was based on HCl or CO_2 ; sample preconcentration-clean up: liquid-liquid extraction were based on organic solvents e.g. dichloromethane, hexane; sample preconcentration-clean up: off-line solid-phase extraction were C_{18} cartridge preconcentration, conditioning with e.g. dichloromethane, acetonitrile or methanol, and eluting with dichloromethane, acetonitrile or ethylacetate. Separation was by High performance liquid chromatographic methods or gas chromatographic methods [45]. All laboratories were requested to perform recovery experiments in order to obtain quantitative information on the efficiency of the extraction procedure. The recovery figures were determined by standard additions. Recovery data ranged from 68 to more than 100%. Relatively low recoveries ($< 80\%$) were accepted when a good reproducibility of the recovery was reported. The data were corrected from the recovery figures.

The determination of these pesticides in freeze-dried water requires the use of complex analytical procedures, sample extraction, clean-up, including numerous sources of possible systematic errors. Different methods used in laboratories working independently and which had previously proven their ability to perform the request task were

TABLE 8.32

CERTIFIED VALUES OF POLAR PESTICIDES IN CRM 606, MASS FRACTIONS EXPRESSED AS mg kg^{-1}

Compound	Certified value	Uncertainty	p
Carbaryl	14	3	10
Atrazine	6.7	0.8	12
Simazine	4.7	0.6	11
Propanil	13.4	1.1	12
Linuron	5.3	0.2	11

applied in the certification. Consequently, it may be accepted that the remaining systematic errors which can not be detected and quantified are randomised. Some participants observed the degradation of carbaryl into β -Naftol. One laboratory had a recovery within the range 29–37%. Therefore, the results were not accepted for certification. For propanil, one laboratory detected an interference which was also present in the blank material. This explained the large standard deviation obtained.

The certified values (in mg kg^{-1}) of the pesticides (atrazine, simazine, carbaryl, linuron and propanil) in freeze-dried water are shown in table 8.32. They correspond to the mean of the means of data sets and were acceptable on technical and statistical grounds.

8.12.4. Participating laboratories

The material was prepared by Chemifarma S.A. in Madrid (Spain) and the Institute of Reference Materials and Measurements (IRMM) in Geel (Belgium). The homogeneity and stability studies were carried out by the Facultad de Química, Universidad Complutense de Madrid (Spain). The following laboratories participated in the certification: Institut de Recherches Hydrologiques, Nancy (France); IFA, Tüln (Austria); Institute of Freshwater Ecology, Dorset (United Kingdom); Bureau de Recherches Géologiques et Minières, Orleans (France); Customs Laboratory, Helsinki (Finland); National Laboratory of Agricultural Chemistry, Uppsala (Sweden); Clyde River Purification Board, Glasgow (United Kingdom); Centro de Investigación y Desarrollo, CSIC, Barcelona (Spain); ESPCI, Paris (France); University of Ioannina, Ioannina (Greece); LARA, Toulouse (France); Antwerp Waterworks (Belgium); Centro de Investigação do Ambiente, Lisboa (Portugal); RIVM, Bilthoven (The Netherlands); Univerddad Complutense, Madrid (Spain).

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*Chapter 9***CRMs for sediment analysis****9.1. TRACE ELEMENTS IN THREE SEDIMENTS****9.1.1. Introduction**

Sediments are currently analysed in environmental monitoring since they provide the 'history' of contaminations in their stratigraphic structure, they are necessary for calculations of the total load of a river as most of the polluting inorganic or organic material are adsorbed to the solid particles and, prior to a decision on the possible deposition of a sediment, the contents have to be determined to estimate the effect of disposal on groundwater and (later) plant life. For this latter purpose very often the aqua regia soluble heavy metal contents are used instead of the total contents. To respond to the needs of sediment CRMs, three materials have been prepared, namely estuarine sediment (CRM 277), lake sediment (CRM 280) and river sediment (CRM 320).

9.1.2. Production of the candidate CRMs

The estuarine sediment was collected in an amount of ca. 650 kg near the Zandvlietlocks in the Belgian part of the Scheldt Estuary using a Van Veen grab. This material was polluted with the effluents of chemical and metallurgical industries. The sediment was oven dried at 105°C in layers of approximately 5 cm, crushed and shipped to the Joint Research Centre of Ispra (Italy) for further processing.

The lake sediment has been taken from Lago Maggiore (Italy) at one of the deepest basins in the northern part of the lake near Cannero (from a depth of ca. 280 m). A modified Eckman grab sampler was used. The sample collected from this depth is typical for a fine particle anaerobic deep-lake sediment. There was no local source of pollution in the vicinity of the sampling area. Immediately after collection, the sample was air-dried, crushed and sieved through a sieve with apertures of 90 µm and set aside for further treatment.

The river sediment was collected from a bank located at 500–700 m of the merging point of the river Toce and Lago Maggiore (Italy). The sample is typical for an anaerobic situation in rivers with prolonged industrial activity (chlorine-alkali plant). It was treated in the same manner as the lake sediment.

Each of the three sediments was homogenised during two weeks in a special polythene-lined mixing drum under dry argon. Following homogenisation, each sediment was bottled (ca. 40 g) in batches of 40. After each batch of bottling the contents of the

drum were mixed again for 30 minutes. One bottle was randomly selected out of each batch of 40 bottles and set aside for the homogeneity study.

The homogeneity of the materials was studied at levels of 50, 100 and 250 mg. INAA analyses were performed on Cu, Cr, Mn, Fe and Zn; simultaneous ICP-AES measurements were performed after pressurised digestion with HNO_3 followed by HF treatment for Al, Ca, Mg, Ni, Pb and V. This procedure of homogeneity testing has been described in more detail elsewhere [1,2]. The results showed that the materials are homogeneous at a level of 100 mg and above [3].

The stability was verified at -20°C , $+20^\circ\text{C}$ and $+40^\circ\text{C}$ over a period of 12 months (0.3, 3, 6 and 12 months) by the determination of Cu and Pb (FAAS), Hg (CVAAS), Cd and Mo (ETAAS), and Fe, Na, Se and Zn (INAA). The apparent variations of the values were rather due to analytical uncertainties than to an instability of the content of a trace element. Even at prolonged storage at $+40^\circ\text{C}$ losses could not be detected [3].

9.1.3. Certification

The techniques of final determination used by the participating laboratories (see section 9.1.4) are summarised in Table 9.1. Pretreatment techniques included digestion with various acids (HNO_3 , HClO_4 , HCl , HF etc.) in a pressurised or atmospheric mode, irradiation with thermal neutrons etc. More details on the techniques used are given in the certification report [3].

The three sediments having relatively high contents of heavy elements, contamination is not likely to occur if appropriate care is paid to obtain reagents of sufficient purity and if the work is carried out in an atmosphere which meets standard requirements of clean work. Materials like these, with a fair abundance of silicates, alumino-silicates (clay-type particles) and organic matter are difficult to digest. Care must be taken that the whole sample is digested. Pressurised digestions with oxidising acids, followed by

TABLE 9.1

SUMMARY OF TECHNIQUES OF FINAL DETERMINATION USED IN THE CERTIFICATION OF TRACE ELEMENTS IN ESTUARINE SEDIMENT CRM 277, LAKE SEDIMENT CRM 280 AND RIVER SEDIMENT CRM 320

Elements	Techniques of final determination
As	HICP, HGAAS, INAA, RNAA, ZETAAS
Cd	DPASV, ETAAS, FAAS, ICP-AES, IDMS, RPAA, ZETAAS
Cr	FAAS, ICP-AES, IDMS, INAA, RPAA, ZETAAS
Cu	DPASV, EDXRF, FAAS, ICP-AES, IDMS, ZETAAS
Hg	CVAAS, HICP, RNAA
Ni	FAAS, ICP-AES, IDMS, ZETAAS
Pb	DPASV, EDXRF, FAAS, IDMS, RPAA, ZETAAS
Sc	ICP-AES, INAA
Se	EDXRF, ETAAS, FLUOR, HGAAS, IDMS, RNAA
Zn	DPASV, EDXRF, FAAS, ICP-AES, IDMS, INAA

repeated additions of hydrofluoric/nitric acid and evaporation to dryness, or lithium-borate fusion were recommended except when elements which could form volatiles compounds were to be determined.

The quantitative recovery of elements such as Cr after an acid digestion was difficult for three reasons: (1) resistance to chemical attack of many inorganic Cr-species (e.g. oxides), (2) a low reaction rate with reagents in solution (owing to large sphere of hydration) causing apparent losses, and (3) losses when evaporating to dryness due to volatilisation of chromyl chloride; the latter could be obviated by removing chloride using sulphuric acid (not perchloric acid) before any Cr can be oxidised to Cr(VI). Resistant clay particles can be attacked using the LiBO_2 -fusion. One ICP-AES set of results was rejected owing to possible formation of chromyl chloride during the pre-digestion step.

It was stressed that these sediments contain considerable amounts of Ca, Sr and Ba, which may form sulphate precipitates, especially when sulphuric acid has been used in the digestion procedure. Formation of such insoluble sulphates does not take place immediately but depends on acidity, time, available surfaces etc. Hence these precipitates are often not detected upon visual inspection after the digestion. However, when they are formed, they may collect elements such as Pb, leading to low recoveries. In many cases, sulphuric acid can be replaced by perchloric acid, although safety precautions may prohibit the application of this acid.

One participant using CVAAS applied a pyrolytic system to release Hg from the sample. This system had given correct results in a number of different samples such as plants, sludges, soils, mussel tissue etc. Nevertheless, the laboratory verified the procedure using a pressurised digestion with nitric acid and microwave heating; comparable results were obtained using the two different procedures [3].

In the case of Pb, a risk exists that, after a digestion with oxidising acids, insoluble Ba- and Sr-sulphates are formed (the samples contain S which upon oxidation yields sulphate, as well as Ba- and Sr-compounds). This formation may occur after removal of excess acids (e.g. by evaporation or neutralisation) or upon dilution, because the HSO_4 -ion is too weak an acid. This sulphate precipitate may collect other ions such as Pb^{2+} and losses may occur owing to coprecipitation. The inorganic fraction of CRM 280 has a higher content of aluminosilicates (clay-type particles) which are difficult to digest but which normally contain some Pb. Results could only be accepted from non-chemical techniques (EDXRF, RPAA) or from a technique which is not sensitive to coprecipitation after the digestion (e.g. IDMS) or from techniques which remove the sulphate (e.g. by evaporation to dryness in the presence of an excess of HClO_4 or slow dry ashing procedure with acidic ashing aids). Because of these reasons, the sample treatment procedures used by some participants were rejected.

The certification of Se in CRM 320 had to be based on fluorescence spectrometry, RNAA and IDMS owing to too low limit of determinations of other techniques (e.g. HAAS, ETAAS) at the time of the exercise.

The determination of Zn by INAA is interfered by Sc (gamma-energies of 1120 and 115 keV, respectively); especially if the content of Sc is somewhat higher than in biota, the deconvolution between the peaks of Sc and Zn is very difficult. For CRMs 277 and 280, INAA results were accepted only if two different deconvolution techniques arrived

at the same results. For CRM 320 where the Sc-peak is 100 times higher than the Zn-peak it was decided not to accept INAA-values as apparently the method was out of control and an acceptable deconvolution technique did not exist.

The certified values are listed in Table 9.2. Indicative values are given in the certification

TABLE 9.2

CERTIFIED CONTENTS OF TRACE ELEMENTS IN CRMS 277, 280 AND 320

	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	p
CRM 277			
As	47.3	1.6	10
Cd	11.9	0.4	15
Cr	192	7	11
Cu	101.7	1.6	16
Hg	1.77	0.06	9
Ni	43.4	1.6	9
Pb	146	3	9
Sc	9.00	0.12	5
Se	2.04	0.18	9
Zn	547	12	16
CRM 280			
As	51.0	2.4	9
Cd	1.6	0.1	14
Cr	114	4	11
Cu	70.5	1.5	16
Hg	0.670	0.019	9
Ni	73.5	2.6	10
Pb	80.2	2.3	9
Sc	12.8	0.7	6
Se	0.68	0.06	8
Zn	291	4	15
CRM 320			
As	76.7	3.4	10
Cd	0.533	0.026	12
Cr	138	7	11
Cu	44.1	1.0	15
Hg	1.03	0.16	9
Ni	75.2	1.4	10
Pb	42.3	1.6	8
Sc	15.25	0.36	6
Se	0.214	0.034	4
Zn	142	3	11

report [3]. In particular, some participants determined the aqua-regia soluble contents of a range of elements of which the data are given in the report.

The comparison of methods for some elements did not allow to detect any bias due to one specific technique since the CVs within one method were systematically larger than those of different techniques (Table 9.3).

9.1.4. Participating laboratories

The material was prepared by the Joint Research Centre of Ispra (Italy); the homogeneity study was carried out by the GSF-Forschungszentrum für Umwelt und Gesundheit in Oberschleißheim (Germany) whereas the stability study was performed by the Energieonderzoek Centrum Nederland in Petten (The Netherlands).

The following laboratories participated in the certification: An Forais Taluntais, Wexford (Ireland); Arbeitsgruppe Analytik und Umwelttechnik HIT, Hamburg (Germany); Bundesanstalt für Materialprüfung und Forschung, Berlin (Germany); Centro di Radiochimica e Analisi per Attivazione, CNR, Pavia (Italy); Delta Institute for Hydrobiological Research, Yerseke (The Netherlands); Energieonderzoek centrum Nederland, Petten (The Netherlands); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); Institut für Anorganische Chemie der Universität Regensburg (Germany); Institute for Soil Fertility, Haren (The Netherlands); Isotopcentralen/ATV, Copenhagen (Denmark); Instituut voor Nucleaire Wetenschappen, Universiteit Gent (Belgium); Risø National Laboratory, Isotope Division, Roskilde (Denmark); Kernforschungsanlage, Institut für Angewandte Physik. Chemie, Jülich (Germany); Laboratoire de Chimie Minérale EHICS, Strasbourg (France); Service Central d'Analyse, CNRS, Vernaison (France); Service de Contrôle des Eaux de Paris, Paris (France); Studiecentrum für Kernenergie SCK/CEN, Mol (Belgium); The Macaulay Land Use Research Institute, Aberdeen (United Kingdom); Universitaire Instelling Antwerpen, Wilrijk (Belgium); Water Research Centre, Medmenham (United Kingdom).

9.2. FLUORINE IN CLAY

9.2.1. Introduction

Clay materials used in the brick industries are currently monitored to control air and groundwater pollution. Some areas suffer from severe pollution by emission of fluorine to the atmosphere and its subsequent transport into the groundwater since clay may contain 200 to 1200 mg kg⁻¹ of F. As an example, the brick and ceramic industry is responsible for 50% of the total fluoride emission in The Netherlands. The emissions are calculated from the difference in the F content of the clay and the final product (brick). In some cases, raw materials are rejected if the fluoride contents are too high which cause an economic concern. As a consequence, many European countries have implemented regulations for the maximum authorised amount in gaseous emission of F from brick industries, which strongly stresses the need for the control of the quality of measurements using representative CRMs.

TABLE 9.3

COMPARISON OF METHODS FOR TRACE ELEMENTS IN CRMS 277, 280 AND 320

CRM	Elem.	Techn. of final determination	CV % between means of lab. with the same technique	CV % between means of diff. techniques
277	As	INAA	5.7	2.2
		HGAAS	3.2	
280		INAA	7.1	
		HGAAS	7.9	
320		INAA	5.0	3.9
		HGAAS	4.7	
277		ZETAAS	6.1	
		FAAS	4.2	
	Cd	DPASV	11.0	2.6
		IDMS	4.3	
280		ZETAAS	16.0	
		DPASV	6.4	
		IDMS	2.0	6.0
320		ZETAAS	6.4	
		DPASV	10.0	
		IDMS	2.1	
277		ICP-AES	3.8	1.5
		FAAS	3.4	
		IDMS	1.8	
		ICP-AES	2.7	
280	Cu	FAAS	3.7	1.3
		IDMS	0.8	
		ICP-AES	1.5	
320		FAAS	5.8	
		IDMS	1.1	1.0
277		ZETAAS	4.4	
		FAAS	5.6	
		IDMS	1.5	
280	Pb	ZETAAS	7.4	2.1
		FAAS	7.6	
		IDMS	1.2	
320		ZETAAS	7.5	
		FAAS	13.0	3.8
		IDMS	1.7	
277		INAA	5.2	
		ICP-AES	5.5	
		FAAS	2.5	1.4
		IDMS	4.4	
280		INAA	1.2	
		ICP-AES	0.6	
	Zn	FAAS	2.6	0.9
		IDMS	0.9	
320		ICP-AES	1.3	
		FAAS	3.6	
		IDMS	1.3	2.7

A group of laboratories has worked in various interlaboratory studies to improve their methods for F determination [4], especially for coal analysis. Methods were improved to such an extent that the certification of fluoride in clay could be envisaged and a CRM was produced in 1994 (CRM 461) [5].

9.2.2. Production of the candidate CRM

The material was collected in the Rhine River, air-dried and further dried at 40°C for 48 h. About 40 kg were milled, homogenised and sieved to obtain particles of less than 250 µm size. The treatment was carried out following the Dutch norm NEN 5751. The material was bottled in brown glass bottles (1200 bottles each containing ca. 30 g).

The homogeneity was verified on intakes of 100 mg. Fluorine determinations were performed by addition of sodium carbonate, melting at 950°C for 30 min and final detection by Ion Sensitive Electrode. The results showed that the material is homogeneous at a level of 100 mg and above.

The stability was verified at -20°C, +20°C and +40°C over a period of 12 months (1, 3, 6 and 12 months). No instability for fluorine could be observed even at prolonged storage at +40°C.

9.2.3. Certification

The techniques used were mainly based on either pyrohydrolysis or melting followed by ISE potentiometry (5 laboratories), fusion followed by IC fluorescence, and fast neutron activation analysis.

At the technical meeting, it was suspected that the results obtained by all the participants were too low due to possible losses of F during the fusion procedure (using sodium carbonate at 950°C whereas F is volatilised from clay materials after 650°C). The participants considered that melting with sodium hydroxide was more adequate for recovering F in this case. An experiment was made by fusing the clay with sodium carbonate at 950°C during 20 min in a furnace tube and determining the F emitted in the headspace. The mean result obtained was about 570 mg kg⁻¹ and an amount corresponding to about 30 mg kg⁻¹ was found to be emitted. After subtraction of the blank value (5 mg kg⁻¹), the actual F content would be about 590 mg kg⁻¹. Another laboratory, however, performed F determinations (using both sodium hydroxide and carbonate) and obtained exactly the same results with both reagents; the procedure was therefore accepted.

One participant repeated its analysis with a higher combustion time and additional water steam and iron phosphate and found higher results than those previously submitted. Fluoride could not be found in the residue (verified by fusion) and was assumed to be lower than the detection limit, i.e. 20 mg kg⁻¹. The three methods used were in good agreement (ISE after combustion, fusion/steam distillation, steam distillation).

An attempt was made to certify chlorine in this material. However, a high spread of results and a bimodal distribution rendered the discussion very difficult, i.e. it was impossible to evaluate why some results were likely to be wrong rather than others and consequently it was agreed that the material should not be certified for chlorine.

The certified value of fluorine in CRM 461 and its uncertainty is $(568 \pm 10) \text{ mg kg}^{-1}$.

9.2.4. Participating laboratories

The coordination of the certification was carried out by KEMA N.V. in Arnhem (The Netherlands). The preparation of the material was performed by the Energieonderzoek Centrum Nederland in Petten (The Netherlands) which also carried out the homogeneity and the stability studies. The following laboratories participated in the certification: Danish Technological Institute, Hasselager (Denmark); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Ruhrkohle AG, Essen (Germany); TÜV, Essen (Germany); Universiteit Ghent, INW, Ghent (Belgium); University of Plymouth, Department of Environmental Sciences, Plymouth (United Kingdom).

9.3. ORGANOTIN COMPOUNDS IN SEDIMENT

9.3.1. Introduction

Butyl- and phenyl-tin compounds, particularly trisubstituted species, are known to be very toxic to marine organisms at very low concentrations [6,7]. Tributyltin (TBT) is released in the marine environment from the leaching of TBT-based antifouling paints used on boats and ships whereas triphenyltin (TPhT) is used both as antifouling agent and herbicide formulations. The monitoring of tin compounds is required by the EC legislation, e.g. under the Council Decisions 75/437/EEC (Marine Pollution from Land-based Sources), 77/585/EEC (Mediterranean Sea) and 77/586/EEC (Rhine River), and the Council Directive 80/68/EEC (Groundwater). The compliance to this legislation and the need for a comparability of data produced worldwide require that the analyses are accurate. However, the methods currently used for the determination of organotin compounds involve various analytical steps such as extraction, derivatisation, separation and final detection which multiply the risks of analytical errors [8], some of these techniques being far from being validated. In order to improve and ensure a good quality control of tin speciation analysis, series of interlaboratory studies (including certifications) have been organised in the past few years.

9.3.2. Preliminary investigations

The programme to improve the quality control of organotin determinations in environmental matrices started in 1988 [9] by a consultation of European experts. It was decided to follow a stepwise approach for the evaluation of the performance of methods used in butyltin analyses. The overall programme consisted in a series of interlaboratory studies which started by an intercomparison on organotins in solutions followed by an intercomparison on a TBT-spiked sediment.

Four solutions (HCl-acidified) containing different possible interferents were used in the first round: solution A with TBTAc only and solutions B, C and D containing

TBTAc along with, respectively Sn(IV) and DBTCl (sol.B), Sn(IV) and MBTCl (sol.C) and TPhTAc (sol.D). The results of this intercomparison were found to be in good agreement which indicated that possible systematic errors did not arise from the techniques of final determination [9].

The second exercise was undertaken in 1989 on the determination of TBT in a spiked sediment (collected in the Lago Maggiore, Italy, and prepared at the EC Joint Research Centre of Ispra) [9]. The results of this interlaboratory trial did not reveal any systematic errors in the different analytical methods compared. The coefficient of variation (CV) obtained between laboratories (25%) was considered to reflect the state of the art at that stage and the group of experts recommended to proceed with the organisation of a certification campaign. The participants recognised, however, that a better agreement should be achieved for certification. No particular source of error due to a method could be detected and the analytical techniques used in this intercomparison were therefore found suitable for certification. The need to allow spikes to equilibrate at least overnight to get a realistic assessment of extraction recoveries was highlighted [9].

These two interlaboratory studies were followed by a tentative certification of butyltin compounds in a harbour sediment (RM 424) and the certification of di- and tributyltin in a coastal sediment (CRM 462) which are described below.

9.3.3. Production of the candidate reference materials

9.3.3.1. Harbour sediment

The harbour sediment (RM 424) was collected in the Sado Estuary (Portugal) in the vicinity of the harbour of Setúbal [10]. The first 10 cm sediment layer was collected with a shovel and stored at ambient temperature in plastic containers. After decantation, the sediment sample was air dried for 7 days at ambient temperature on a cotton sheet in a well-ventilated room, and stored at -20°C in polythene bags. The material (about 180 kg dry mass) was then stored in ice boxes for transportation to the Institute for Reference Materials and Measurements, IRMM (Geel, B). The material was dried at 80°C in air for 100 h; it was then ground and sieved through a sieve of $75\text{ }\mu\text{m}$ mesh size. Before homogenisation, the powder obtained was sterilised by heating at 120°C for one hour and homogenised in a mixer for one hour. The material was finally stored in 60 mL well cleaned brown glass bottles with polyethylene inserts and plastic screw caps, each containing ca. 25 g of powder.

The moisture content of the material was determined by Karl Fischer titration on ten samples selected during the bottling procedure. The mean moisture mass fraction measured was $(2.24 \pm 0.36)\%$.

The between bottle homogeneity was verified by the determination of TBT on intakes of 2 g. The technique used for this study was hydride generation/cold trapping-gas chromatography/atomic absorption spectrometry. No significant differences were observed between the CV of the method ($8.7 \pm 2.8\%$) and the CV within-bottles ($8.2 \pm 1.8\%$). The CV between-bottles appeared to be significantly higher than the CV within-bottles ($18.3 \pm 2.9\%$); however, the value found corresponded to determinations

performed over a period of six months and, therefore, included the long term reproducibility of the analytical method used, which was realistic at this mass fraction level. Any inhomogeneity of the material would have actually been detected in the stability study.

The stability of the TBT content was tested at respectively -20°C , $+20^{\circ}\text{C}$ and $+40^{\circ}\text{C}$ over a period of 12 months. In the case of storage at $+40^{\circ}\text{C}$, slight TBT losses were suspected to occur after 6 months storage. Although the RM was shown to be stable at $+20^{\circ}\text{C}$, a recommendation was given to store this material at $+4^{\circ}\text{C}$ for long term storage.

9.3.3.2. Coastal sediment

Heating procedures were tested on a test sediment portion to investigate the feasibility of stabilising the material and the possible effects on the stability of butyltin compounds. The aim of this study was to stop possible microbial activities (risk of microbial degradation of TBT) by using a heating procedure which would preserve the organotin contents of the sediment. A small amount of sediment sample was collected in the Arcachon Bay (F) and air dried at ambient temperature (ca. 20°C) on a polythene film in a clean room for 7 days. The material was ground in a porcelain crucible and sieved at $80\text{ }\mu\text{m}$ mesh. The powder obtained was homogenised manually and bottled in 20 glass bottles each containing ca. 50 g. After bottling, 5 flasks were wrapped in an aluminium film to protect them against light and were placed in a cupboard at ambient temperature and kept as test samples. The other bottles were treated as follows:

- 5 bottles heated at 80°C for 6 h;
- 5 bottles heated at 120°C for 2 h;
- 5 bottles heated at 120°C for 6 h.

The effect of heat on butyltin stability was assessed by determining TBT in duplicate in each bottle. The determination was carried out by acetic acid extraction, hydride generation (using NaBH_4), cryogenic condensation in a U-tube filled with chromatographic material (Chromosorb GAWHP 80–100 mesh) followed by thermal separation (based on the different organotin hydride sublimation points) and AAS detection in a quartz furnace electrically heated to 950°C . A statistical treatment (F-test) did not reveal any significant difference between the sets of results. It was therefore concluded that the heating procedures applied did not entail significant losses of TBT by degradation. Previous results obtained at the University of Pau had, however, shown that drying at 80°C for an overlong period (e.g. overnight) could induce degradation of TBT. Hence, the procedure recommended for the preparation of the candidate CRM was drying at 60°C for 48 h, grinding and sieving, followed by heat-sterilisation at 120°C for 2 h.

The candidate certified reference material (CRM 462) was collected in the southern part of the Arcachon Bay (France) in a small harbour (Larros) [11]. The first 10 cm sediment layer was collected with a shovel and stored in plastic containers. After decantation, the sediment sample was air dried for 7 days at ambient temperature on a cotton sheet in a well-ventilated room, and stored at -20°C in polythene bags. The material (about 180 kg dry mass) was then stored in ice boxes for transportation to IRMM.

The material was dried at 55°C in air for 100 h. The moisture content determined

at this stage was less than 3%. The material was then sieved through a sieve of 1 mm mesh size and finely ground using a jet mill grinding device with a classifier. This procedure allowed a powder with a closely defined particle size distribution (less than 75 μm) and a sharp maximum size limitation (no oversized particles) to be obtained. The material was then sterilised by heating at 120°C for two hours, homogenised in a mixer for two hours and finally stored in 60 mL well cleaned brown glass bottles with polyethylene inserts and plastic screw caps, each containing ca. 25 g of powder.

The moisture content of the material was determined by Karl Fischer titration on ten samples selected during the bottling procedure. The mean moisture mass fraction measured was $(2.70 \pm 0.40)\%$.

The between bottle homogeneity was verified by the determination of DBT and TBT on intakes of 1 g. The within-bottle CV was very close to the CV of the method and, therefore, no inhomogeneity of the material was suspected.

The stability of the material was tested in the same way as the RM 424. For TBT at +20°C, the content decreased after three months of storage and stabilised afterwards. This decrease was likely to be due to a degradation of TBT directly to MBT as previously observed, because of a corresponding increase of MBT content after 3 months (from 66 to 93 $\mu\text{g kg}^{-1}$ as Sn). In the case of storage at +40°C, both DBT and TBT displayed strong losses after 3 months which was likely due to a degradation of these compounds to MBT and inorganic tin.

On the basis of the results, it was concluded that:

- DBT is stable at +20°C in the dark;
- TBT displayed losses at the start of the storage period but the content stabilised after 3 months. It is hence concluded that TBT remained stable at +20°C in the CRM in the dark; however, in order to avoid any risk of organotin degradation during long term storage, it was decided to store the material at +4°C in the dark;
- both DBT and TBT are unstable at +40°C. However, the two compounds are stable at this temperature for at least one month [11], which indicates that the material could be transported safely under extreme conditions.

9.3.4. Certification

As mentioned in the introduction, methods used in tin speciation are generally composed of a succession of analytical steps (extraction, derivatisation, separation and detection) which vary drastically from one laboratory to another. The variety of methods used in the certifications is summarised in Table 9.4.

9.3.4.1. Harbour sediment

A very large scatter of data was obtained for TBT which ranged from less than 10 $\mu\text{g kg}^{-1}$ of TBT to more than 150 $\mu\text{g kg}^{-1}$. Detailed discussions were necessary to explain the sources of discrepancies. Some laboratories reported not detected values ranging from less than 15 $\mu\text{g kg}^{-1}$ to less than 60 $\mu\text{g kg}^{-1}$.

The laboratories reported their results of extraction recovery which were generally acceptable (from 80 to 100%). It was assumed that the main problems were not due to

TABLE 9.4

TECHNIQUES USED IN Sn-SPECIATION

Techniques used in the certification

Acetic acid extraction, derivatization with NaBH_4 , cryogenic trapping in an U-tube filled with chromatographic material, QFAAS detection

NaOH /methanol extraction, back-extraction into hexane, derivatization with NaBH_4 , capillary GC followed by FPD

Extraction with HCl /toluene, derivatization with NaBEt_4 , capillary GC followed by FPD
Supercritical fluid extraction (CO_2 and methanol), Grignard derivatization, capillary GC followed by FPD

Addition of HBr /water mixture, tropolone/pentane extraction, Grignard pentylation, capillary GC followed by FPD

Extraction with acetic acid/DDTC in pentane, Grignard pentylation, capillary GC followed by QFAAS detection

Diethylether/ HCl extraction in tropolone, Grignard pentylation, capillary GC followed by MS detection

Acetic acid extraction, toluene back-extraction, HPLC separation followed by ICP-MS detection

Leaching with HCl , centrifugation of the slurry, dichloromethane extraction, scanning of alternative current polarograms

extraction but to possible interferences in the derivatisation and/or in the detection step. Aromatic compounds were suspected to have inhibited the hydride generation but not the ethylation reaction; methanolic- HCl extraction did not extract the oil present in the sample but a 35% suppression of hydride generation was still observed. It was felt, however, that the interferences were rather occurring at the atomisation stage rather than in the hydride generation step; high contents of Fe and Cr could have led to a suppression of the TBT signal, as demonstrated with spiking experiments. An extensive study was carried out by the University of Bordeaux on interference effects from inorganic (metals) and organic substances (organic solvent, PCB, pesticides, n-alkane, humic substances) on the yield of hydride generation. The major effects observed were mostly linked to high levels of trace metals; organic compounds had generally negligible effects on the signal suppression [12].

The methods using hydride generation and AAS as final determination were, however, in considerable difficulty with this complicated matrix, due to unknown interferences either at the derivatisation step or in AAS detection, or determination limits that were too low. As observed by the participants, the laboratories using gas chromatographic separation and detection either by FPD or MS tended to agree which would confirm that these methods would be more suited to the determination of TBT in this particular material.

Although the analytical methods involving hydride generation were successfully applied in the interlaboratory study on TBT in spiked sediment [9], it was concluded that the low TBT mass fractions and the complicated matrix did not allow the use of

these methods for an accurate TBT determination in this material. It was stressed that, although certification was contemplated at the start of the interlaboratory study, this material would probably not be suited as a CRM for the following reasons:

- the low content of TBT is very close to the limits of determinations of most of the techniques used in this exercise;
- the high level of interferences makes this material difficult to analyse which renders quite doubtful its use as a CRM for routine analysis;
- both the low TBT content and the complicated matrix do not resemble sediments usually analysed for TBT monitoring. This unrepresentativeness is another justification for not proposing this material as a CRM.

Consequently, the RM 424 was not certified and was considered as a research material for laboratories willing to evaluate techniques such as e.g. GC/FPD, GC/MS or HPLC/ICPMS. The reference value (unweighted mean of 8 accepted sets of results) and its standard deviation is $(20 \pm 5) \mu\text{g kg}^{-1}$ as mass fractions (based on dry mass) of TBT cation.

9.3.4.2. Coastal sediment

Poorer extraction recoveries for TBT in CRM 462 were often observed in comparison to recoveries obtained from other sediment materials. Some laboratories found lower recoveries if the spike was allowed to equilibrate longer. The previous interlaboratory exercise on TBT-spiked sediment had identified the need to allow spikes to equilibrate at least overnight to get a realistic assessment of extraction recoveries.

The evaluation of polarographic methods showed that there were major problems with this sample. The surfactants present made it difficult to detect TBT and polarography was, therefore, a method not recommended for this certification.

In some cases, the uncertainty on extraction recoveries was not taken into account in the overall uncertainty of the laboratory means which explained the occurrence of small standard deviations.

For dibutyltin, the overlap obtained between the different sets of results and the range of techniques used was found satisfactory and, as no doubts were thrown on the results presented, it was agreed to certify the DBT content.

In the case of monobutyltin, a high scatter of results was observed for MB, which prevented certification. Many laboratories reported problems in the extraction step. The addition of complexing agents such as diethyldithiocarbamate or sodium dithiocarbamate may enhance the extraction recovery. However, the addition of complexing agents was found to prevent the hydride generation of a volatile species for MBT. Considering the high spread of results (ranging from 13 to $244 \mu\text{g kg}^{-1}$ as MBT) and the doubts still remaining on the different techniques used, it was decided not to give any indicative values for MBT which could be misused by laboratories. Further efforts should be made to improve the state of the art of MBT determinations before contemplating certification of this compound at the $\mu\text{g kg}^{-1}$ level. A similar lack of agreement on MBT results has already been shown in an interlaboratory study of analytical techniques [13]; this study confirmed, however, that the techniques used for DBT and TBT determinations were satisfactory.

The two compounds were certified as mass fractions ($\mu\text{g kg}^{-1}$) of, respectively, $\text{Sn}(\text{C}_4\text{H}_9)_3^+$ with a value of $(70 \pm 14) \mu\text{g kg}^{-1}$ as TBT and $\text{Sn}(\text{C}_4\text{H}_9)_2^{2+}$ with a value of $(128 \pm 16) \mu\text{g kg}^{-1}$ as DBT.

9.3.5. Further developments

Recent findings have shown that the certified value of TBT in the CRM 462 has decreased by ca. 20% over a long-term period (36 months storage at $+4^\circ\text{C}$). This has justified that the material be withdrawn from the market and that a new homogeneity and stability study be undertaken. A re-certification of DBT and TBT in CRM 462 has been conducted in 1997 by the IRMM. The results show that materials certified for their butyltin contents should imperatively be stored at -20°C in the dark. The new certified values are $(54 \pm 15) \mu\text{g kg}^{-1}$ as TBT and $(68 \pm 12) \mu\text{g kg}^{-1}$ as DBT [14].

A complementary project has started in 1997 by an interlaboratory study on butyl- and phenyl-tin compounds in a freshwater sediment reference material. A certification campaign has been conducted in 1998 and the certification of the six compounds (mono-, di- and tri-butyl and phenyltins) is promising [15]. All precautions have been taken to ensure the material stability (storage at -20°C).

9.3.6. Participating laboratories

The two sediment samples used as candidate CRMs were collected by the Université de Pau (France) and prepared at the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). The verification of the homogeneity and stability was carried out by the Université de Pau. The following laboratories participated in the certification campaigns on sediment: CIBA-GEYGI, Bensheim (Germany); CID-CSIC, Department of Environmental Chemistry, Barcelona (Spain); ENEA, Divisione di Chimica Ambientale, Rome (Italy); Ministry for Agriculture Fisheries and Food, Burnham-on-Crouch (United Kingdom); Universiteit Antwerp, Departement Scheikunde, Antwerp (Belgium); Université de Bordeaux I, Laboratoire de Photophysique et Photochimie Moléculaire, Talence (France); Université de Pau, Laboratoire de Chimie Analytique, Pau (France); University of Plymouth, Department of Environmental Sciences, Plymouth (United Kingdom); Universidad de Sevilla, Departamento de Química, Sevilla (Spain)

9.4. METHYL MERCURY IN SEDIMENT

9.4.1. Introduction

The lack of knowledge of toxical impact of methyl mercury (MeHg) in sediment (e.g. on filter-feeding organisms) and the need to better understand the environmental pathways justifies the monitoring of this compound in various matrices (biota, water and sediment). Legislation on MeHg within the European Union, e.g. in food (national regulations) or water (EC Directives), requires that the determinations are of proven

quality. A certification campaign on MeHg in sediment has been recently organised by the International Atomic Energy Agency (IAEA, Vienna), which showed a reasonable agreement between techniques (coefficient of variation between laboratory means of ca. 14% for a MeHg level of $5.46 \mu\text{g kg}^{-1}$) and enabled a CRM to be produced [16]. Upon request of a consortium of laboratories from different EU and EFTA (European Free Trade Association) Member States, the Measurements and Testing programme (formerly BCR) has organised an interlaboratory study for the evaluation of the method performance for the determination of MeHg in a highly contaminated sediment which was successfully concluded in 1995 [17]. The project was pursued by the preparation of a sediment candidate reference material (CRM 580) which was certified for its content of total mercury and methylmercury.

9.4.2. Interlaboratory study

The feasibility of preparation and certification of a sediment containing high levels of methyl mercury has been tested, including the collection, preparation, homogenisation, stabilisation by gamma-irradiation, homogeneity and stability studies of a test material, followed by an interlaboratory study with a group of EU expert laboratories [17]. This interlaboratory project enabled to confirm the feasibility of preparation of a candidate CRM and to detect and remove some systematic errors in the methods used in the exercise. The CV obtained for the mean of laboratory means was ca. 16% at a level of $(53.1 \pm 8.5) \mu\text{g kg}^{-1}$ as MeHg. Besides errors due to an apparent lack of quality control for some laboratories, a systematic bias was suspected to occur in relation to the distillation procedure; hence, recommendations were given that this method be carefully checked by spiking experiments for further use in the certification campaign [17]. The outcome of the technical scrutiny was a clear illustration of the effects and importance of participating in interlaboratory studies; indeed, most of the laboratories from which the data were selected had participated previously in the stepwise interlaboratory programme on MeHg in solutions and biological samples, whereas most of the other laboratories for which sources of error were identified participated in such an exercise for the first time [17]. This interlaboratory study gave encouragement to the latter laboratories to improve their methods which were further tested in the course of the certification described in this section; it clearly stressed, of course, the importance of method validation, including recovery tests, to obtain accurate results [18].

9.4.3. Production of the candidate CRM

The sediment was collected in the Ravenna Lagoon (Italy) close to a petrochemical plant water discharge. A batch of ca. 250 kg was collected from a boat, using a grab collecting the 30–40 cm top sediment layer. The wet material was sieved to pass apertures of 2 mm and air-dried at 25°C in a drying chamber. The moisture content was monitored during the drying process; when a constant moisture content was reached (3.5%), the material was manually crushed and sieved again at 2 mm to remove coarse particles (the fraction higher than 2 mm was discarded). The resulting material was stored in polyethylene bags and transported to the Joint Research Centre of Ispra (Italy).

The material was passed through a tungsten carbide-bladed hammermill and sieved to pass apertures of 90 μm in order to ensure a good homogenisation. The <90 μm fraction was collected in a PVC mixing drum (filled with dry argon) and homogenised for 14 days at about 48 rpm. The bottling procedure was performed manually: a first series of 20 bottles was filled and immediately closed with screw caps and plastic inserts. Series of 20 bottles were hence successively filled, alternating with re-mixing of the powder for 2 minutes. All bottles were stored at ambient temperature in the dark. Around 1100 bottles were prepared, each containing ca. 40 g.

The optimal stabilisation procedure (by gamma-irradiation) investigated on the test material in the interlaboratory study [17] was used on the candidate CRM. The optimal gamma-ray irradiation dose was found to be 8 kGy upon which the sterilisation of the sediment was achieved without affecting the methylmercury content [17,19].

The homogeneity of the material was verified by the determination of total mercury and methylmercury on sample sizes of 50, 100 and 250 mg. For the determination of total mercury, 100 mg dry sediment was mineralised by addition of aqua regia. The final determination was performed by cold vapour atomic absorption spectrometry (CVAAS) after SnCl_2 reduction. Methylmercury was determined by capillary gas chromatography (CGC) followed by hydride generation atomic absorption spectrometry (HGAAS). The within-bottle CV for total mercury was very close to the CV of the method; with respect to methyl mercury, the higher value of the within-bottle CV in comparison to the method CV was related to the additional uncertainty of extraction which is not taken into account in the method CV calculation (analysis of extracts). On the basis of these results, no inhomogeneities of the material were suspected. It was concluded that the material is suitable for use as a CRM and is homogeneous at an analytical portion of 250 mg and above for total and methyl mercury.

The stability of the material was tested at -80°C , $+20^\circ\text{C}$ and $+40^\circ\text{C}$, respectively, over a period of 15 months and total mercury and methylmercury were determined at regular intervals during the storage period. No instability of the material could be demonstrated.

9.4.4. Certification

A brief description of the methods used is given in Table 9.5; additional details on these methods may be found in the certification report [19].

For total mercury, two sets of low results were rejected owing to the suspicion of mercury losses; the laboratories concerned had experienced the same difficulty in the interlaboratory study and were encouraged to investigate the source of error leading to biased results. The certified value is mainly based on results obtained by CVAAS as final determination (except one set by CVAFS and two sets by ICPMS); however, the pretreatment techniques were widely different from one laboratory to another.

The determination of MeHg in sediment is prone to a range of possible sources of errors that have to be carefully controlled, e.g. incomplete extraction, incomplete derivatisation or distillation, interferences in detection etc. The first aspect considered in the technical discussion was related to the verification of calibrants. Most of the participants verified their calibrant using alternative calibrant solutions; other participants

TABLE 9.5

SUMMARY OF METHODS USED IN THE CERTIFICATION OF CRM 580

Techniques used in the certification

Digestion of 200 mg with a HNO_3/NaCl mixture, followed by gold preconcentration and determination of total Hg by CVAAS (Lab.01)

Total Hg determined by cold vapour atomic fluorescence spectrometry (CVAFS) after pressurized digestion with H_2SO_4 , addition of BrCl , reduction with SnCl_2 and gold preconcentration. For MeHg, 200 mg pre-treated by addition of $\text{H}_2\text{SO}_4/\text{KCl}$, water-steam distillation, ethylation with NaBEt_4 in acetate buffer solution, separation by capillary gas chromatography, followed by CVAFS detection (Lab.02)

Total Hg determined by CVAAS after pressurized digestion with $\text{H}_2\text{SO}_4/\text{HNO}_3$, reduction with SnCl_2 and gold preconcentration. For MeHg, 2000 mg pre-treated by addition of HCl and toluene, back-extraction with cysteine acetate, and re-extraction into toluene, separation by capillary gas chromatography, followed by electron capture detection (Lab.03)

500 mg extracted by supercritical fluid extraction with CO_2 , elution with toluene, followed by Grignard derivatization (n-butyilmagnesium chloride), separation by capillary gas chromatography, followed by microwave induced plasma-atomic emission spectrometric (MIP-AES) detection (Lab.04)

Total Hg determined by CVAAS after pressurized digestion with $\text{H}_2\text{SO}_4/\text{HNO}_3$ in microwave oven, and reduction with SnCl_2 . For MeHg, 250 mg pre-treated by addition of $\text{H}_2\text{SO}_4/\text{NaCl}$, water steam distillation, addition of acetate buffer, and complexation with sodium pyrrolidine dithiocarbamate, separation by high performance liquid chromatography after preconcentration on a C18 column, followed CVAAS detection (Lab.05)

500 mg digested with HNO_3 in a microwave oven, followed by a derivatization with NaBEt_4 , and cryogenic trapping, separation was by packed-column gas chromatography, followed by quartz furnace atomic absorption spectrometric (QFAAS) detection (Lab.06)

Total Hg determined by CVAAS after pressurized digestion with HNO_3/HCl , and reduction with SnCl_2 (Lab.07)

Total Hg determined by CVAAS after digestion with HNO_3 for 4 h at 80°C , and reduction with SnCl_2 . For MeHg, 1000 mg was pre-treated by addition of HCl and toluene, back-extraction with thiosulphate, addition of acetate buffer, complexation and on-line oxidation (after HPLC), and reduction with SnCl_2 , separation was by high performance liquid chromatography (HPLC), followed by CVAAS detection (Lab.08)

Total Hg determined by inductively coupled plasma mass spectrometry (ICPMS) after microwave digestion with HNO_3 and reduction with SnCl_2 (Lab.09)

Total Hg determined by CVAAS after digestion with HNO_3 for 4 h at 80°C and reduction with SnCl_2 . For MeHg, 500 mg pre-treated by addition of HCl and extraction into toluene, separation was by capillary gas chromatography, followed by electron capture detection (Lab.10)

Total Hg determined by CVAAS after microwave digestion with HNO_3/HCl and reduction with SnCl_2 . For MeHg, 200 mg pre-treated by addition of $\text{H}_2\text{SO}_4/\text{HCl}$, water steam distillation, and NaBEt_4 derivatization, separation was by capillary gas chromatography, followed by CVAAS detection (Lab.11)

Total Hg determined by CVAAS after digestion with $\text{H}_2\text{SO}_4/\text{HCl}$ and reduction with SnCl_2 . For MeHg, 200 mg pre-treated by addition of $\text{H}_2\text{SO}_4/\text{NaCl}$, toluene extraction, addition of thiosulphate solution and NaBEt_4 derivatization, separation was by capillary gas chromatography, followed by CVAAS detection (Lab.12)

Total Hg determined by CVAAS after digestion with HNO_3 for 3 h under reflux and addition of H_2O_2 . For MeHg, 250 mg pre-treated by addition of HCl , toluene extraction, clean-up with cysteine solution and back-extraction into toluene, separation was by capillary gas chromatography, followed by CVAAS detection (Lab.13)

Total Hg determined by ICPMS after microwave digestion with HNO_3/HCl . For MeHg, 200 mg pre-treated by addition of $\text{H}_2\text{SO}_4/\text{HCl}$, water steam distillation, addition of acetate buffer solution, SPDC complexation, toluene extraction, UV-irradiation and NaBH_4 reduction after HPLC, separation was by high performance liquid chromatography, followed by ICPMS detection (Lab.14)

Total Hg determined by CVAAS after digestion with HNO_3 for 4 h at 80°C and reduction with SnCl_2 (Lab.15)

preferred to check their techniques using a certified reference material of sediment (as the one produced by the IAEA [16]).

Discussions arose on the verification of the extraction recoveries. At present, there is no standardised procedure to check the extraction efficiency; the methods used are briefly summarised above, showing that they differed widely from one laboratory to another. The participants recognised that it would be necessary to find out the most suitable recovery test to propose a standardised procedure in order to avoid possible discrepancies; the technique which was most supported was standard addition (e.g. three levels on wet sediment), equilibrating the spiked mixture overnight.

High results were observed with a technique involving distillation and UV-destruction (separation of MeHg by water steam distillation, removal of inorganic mercury by anion exchange resin, decomposition of MeHg to ionic mercury by UV irradiation and detection by CVAAS). Although the results were confirmed by HPLC, it was suspected that this technique did not remove all inorganic mercury. This doubt had already been expressed at the first interlaboratory study [17] and the set was not accepted for certification.

The careful verification of the efficiency of distillation procedures (as recommended in the interlaboratory study) demonstrated that this method was in good agreement with alternative techniques using various types of extractions. This is shown in Figure 9.1 where the results of laboratories using distillation (Labs. 02, 11, 05 and 14) are in

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

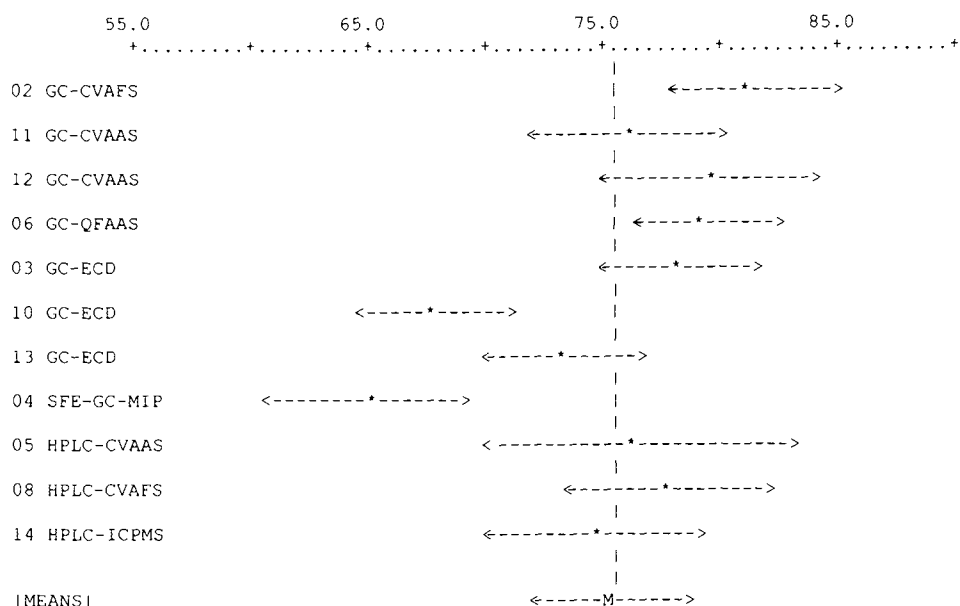


Fig. 9.1. Methyl mercury in $\mu\text{g kg}^{-1}$ (as MeHg)

good agreement with the other sets of results. Doubts expressed on distillation-based procedures in the frame of the Conference 'Mercury as a Global Pollutant' [20] should hence be considered with caution since distillation-based techniques prove to be accurate when applied with a thorough quality control.

Another set of high results was also rejected. The results were obtained by hexane extraction, derivatisation with NaBEt_4 , CGC separation and MIP-AES detection (CGC-MIP). Although this technique was recognised to be suitable for MeHg determination, it appeared that its application in the laboratory was not sufficiently under control to produce accurate results.

Lab. 04 experienced problems of elution from the column, which justified the first data of the set to be withdrawn. The SFE technique gave a rather low recovery which was nevertheless accepted for certification. The laboratory had submitted a second set of data obtained by distillation and CGC-MIP which was on the low side; the efficiency of the distillation was shown to be much lower (70%) than the other laboratories using the same technique which justified the data to be withdrawn.

One laboratory used a biological CRM for calibration, which could obviously not be accepted for certification. The set of data was rejected.

The sets of results accepted after technical scrutiny have been submitted to a series of statistical tests which are fully described in the certification report [19].

The certified values and their uncertainties (given as mass fractions based on dry mass) are, respectively, $(132 \pm 3) \text{ mg kg}^{-1}$ for total Hg (as Hg) and $(75.5 \pm 3.7) \mu\text{g kg}^{-1}$ for methylmercury (as CH_3Hg^+).

9.4.5. Participating laboratories

The certification was coordinated by the Studio di Ingegneria Ambientale, Milano (Italy). The sample preparation was carried out by the Joint Research Centre of Ispra (Italy) and Ecoconsult, Gavirate (Italy). The homogeneity and stability studies were performed by the Presidio Multizonale di Prevenzione' laboratories of La Spezia and Venice (Italy). The following laboratories participated in the certification: ENEA, Divisione Chimica Ambientale, Roma (Italy); GKSS Forschungszentrum, Geesthacht (Germany); Kernforschungsanlage, Inst. Angewandte Physikalische Chemie, Jülich (Germany); Presidio Multizonale di Prevenzione, Lab. Chimico, La Spezia (Italy); Presidio Multizonale di Prevenzione, Sezione Chimico Ambientale, Venezia (Italy); rivo-dlo, Ijmuiden (The Netherlands); Service Central d'Analyse, CNRS, Vernaison (France); Swedish Environmental Research Institute, Göteborg (Sweden); Universität Bayreuth, Inst. Für Terrestrische Ökosystemforschung, Bayreuth (Germany); Universität Heidelberg, Institut für Sedimentforschung, Heidelberg (Germany); Université de Bordeaux I, Lab. Photochimie Moléculaire, Talence (France); Univ. de Santiago de Compostela, Depto. Química Analítica, Santiago (Spain); University of Umeå, Dept. of Analytical Chemistry, Umeå (Sweden); Vrije Universiteit Amsterdam, Inst. voor Milieuvraagstukken, Amsterdam (The Netherlands); Vrije Universiteit Brussel, Lab. Analytical Chemistry, Brussels (Germany)

9.5. EXTRACTABLE TRACE ELEMENTS IN SEDIMENT

9.5.1. Introduction

Sequential extraction schemes have been designed for the determination of binding forms of trace metals in sediment [21] and increasingly used over the last ten years. The lack of uniformity of these schemes, however, did not allow the results to be compared worldwide or the procedures to be validated which led to critical comments [22]. Indeed, the results obtained e.g. by sequential extraction are operationally defined, i.e. the 'forms' of metals are defined by the determination of extractable elements using a given procedure. Therefore, the significance of the analytical results is related to the extraction scheme used.

Owing to the likelihood of the many sources of pitfalls which may occur, a programme was launched, aiming to harmonise sequential extraction schemes for the determination of extractable trace metals in sediment which consisted in two interlaboratory studies followed by a certification campaign on extractable trace metals in a sediment candidate reference material, CRM 601.

9.5.2. Interlaboratory studies

A three step extraction procedure was designed based on acetic acid extraction (step 1), hydroxylammonium chloride extraction (step 2) and hydrogen peroxide/ammonium acetate extraction (step 3). This scheme (described elsewhere [22]) was tested in two interlaboratory trials on Cd, Cr, Cu, Ni, Pb and Zn on freshwater sediment reference materials [23,24], the results of which are summarised in Table 9.6.

The effects of shaking type and speed or room temperature on the spread of results were thoroughly discussed. Changes due to temperature effects were not noticeable. However, it was found more difficult to standardise the shaking procedures. Six laboratories used end-over-end shaker whereas the other laboratories used a horizontal shaker. No effect of shaker type was suspected in this case; however, effects of shaking speed were suspected as lower results were generally obtained at a speed of less than 40 rpm, whereas higher results corresponded to speeds of up to 150 rpm. The need of maintaining the sediment in suspension during shaking was found to be highly critical. The proposal to use a glass ball during shaking was not accepted as it would lead to possible grinding effects. From these results a study of the influence of shaking type and speed was carried out which demonstrated that the copper extracted in this step using an end-over-end shaker, operated at 30 rpm was 20% higher than when using an horizontal shaker operating a 130 rpm. The participants stressed once more that it is of paramount importance to verify that the sediment is continually in suspension during the extraction. If this is not verified, the shaking speed should be adapted in order to ensure a continuous suspension of the mixture. Step 3 was considered to be critical in the presence of high amounts of organic matter (which is the case of the sample used in the second exercise) as the incomplete destruction of organic matter as well the difficulty in oxidising sulphide, may be the source of a high uncertainty which could explain the spread of results.

TABLE 9.6

RESULTS OF THE FIRST AND SECOND INTERLABORATORY EXERCISES (STEPS 1, 2 AND 3). THE TABLE LISTS THE MEAN OF THE LABORATORY MEANS ALONG WITH THE STANDARD DEVIATION (SD) AND THE COEFFICIENT OF VARIATION (CV) OBTAINED. THE TRACE METAL CONTENTS ARE GIVEN IN mg kg^{-1}

Steps	First round-robin			Second round-robin		
	Mean	SD	CV	Mean	SD	CV
Cadmium						
1	7.18	0.81	11.3	0.18	0.02	8.5
2	3.41	0.63	18.5	0.08	0.02	16.9
3	1.03	0.20	20.1	1.02	0.17	16.6
Chromium						
1	1.36	0.20	14.7	1.28	0.41	32.0
2	3.29	1.07	32.5	1.21	0.31	25.6
3	76.3	10.4	13.6	866	126	14.5
Copper						
1	3.69	0.76	20.6	0.23	0.08	34.8
2	3.13	1.96	20.1	0.53	0.33	62.3
3	63.4	13.2	20.8	90.1	7.6	8.4
Nickel						
1	9.76	4.36	44.7	13.0	2.08	16.0
2	5.79	1.54	26.6	1.80	0.23	12.7
3	10.2	3.32	32.5	15.2	2.3	15.2
Lead						
1	5.06	2.50	49.4	0.30	0.17	56.7
2	11.0	8.87	80.6	0.14	0.09	64.3
3	6.93	4.78	69.0	47.7	7.6	15.9
Zinc						
1	262	35.1	13.4	93.9	16.9	18.0
2	140	34.2	24.4	79.7	16.9	21.2
3	89.7	9.14	10.2	676	44	6.5

In some cases, it was difficult to obtain a 'cake' after centrifugation and, consequently, fine particles were still present in solution which created problems in ICP analysis (nebuliser clogging). In these circumstances, a filtration step was necessary. Filtration was not recommended to be included in the protocol; however, when ICP is used, the participants strongly recommended filtration at $0.45 \mu\text{m}$ for ICP users.

Measurements were suspected to be affected by iron interferences and calibration by standard addition was strongly recommended for ETAAS.

The second interlaboratory exercise on extractable trace metals in sediment showed a consequent improvement in comparison with the results of the first exercise.

Furthermore, these collaborative efforts allowed the sequential extraction procedure to be slightly improved by minor amendments [24].

9.5.3. Production of the candidate reference material

The candidate reference material has been collected from different sampling sites of Lake Maggiore (Italy). Sampling operations were performed using grab collectors. The wet sediment was passed through a 2 mm sieve in order to remove stones and other materials extraneous to sediments. The sieved sediment was placed on stainless-steel free trays and exposed at ambient air temperature for drying, turning the lumps from time to time to accelerate the drying process.

The dry sediment with a mean moisture content of 3.5% (calculated by drying a separate portion of sediment at 105°C for 3–4 h) was passed through a tungsten carbide-bladed hammermill and sieved to pass apertures of 90 µm. The <90 µm fraction was collected in a PVC mixing drum (filled with dry argon) and homogenised for 14 days at about 48 rpm.

Bottling operation was carried out after a verification of the bulk homogeneity by XRF. The bottling procedure was performed manually: after an additional period of mixing of 2 days, a first series of 20 bottles was filled and immediately closed with screw caps and plastic inserts. Series of 20 bottles were hence filled, alternating with re-mixing of the powder (2 minutes). The bottles were stored at ambient temperature.

For the homogeneity and stability studies, the trace element contents (Cd, Cr, Cu, Ni, Pb and Zn) were determined by flame atomic absorption spectrometry (FAAS) or electrothermal atomic absorption spectrometry with Zeeman background correction (ZETAAS), strictly following the sequential extraction procedure. Differences between the within-bottle and between-bottle CVs observed for the step 2 were considered to be rather an analytical artefact than an indication of inhomogeneity which would have been reflected in the spread of results submitted in the certification. The material is then considered to be homogeneous for the stated level of intake (1 g).

The stability of the extractable trace element contents was tested at –20, +20 and +40°C during a period of 12 months and the extractable contents of Cd, Cr, Cu, Ni, Pb and Zn were determined (in five replicates) after 1, 3, 6 and 12 months. The detection techniques used were the same as in the homogeneity study. In general, the stability of the extractable trace metal contents was found to be suitable for certification; some risks of instability were, however, suspected at 40°C due to possible changes in the extractability of some elements (e.g. Cu and Pb); these changes induced by the high storage temperature could be related to changes in the status of the organic matter or in the crystallographic compounds of Fe or Mn. Hence, it is recommended to avoid storage at temperature above 20°C.

9.5.4. Certification

The techniques of final determination used to determine the extractable trace element contents were FAAS, ETAAS, ICPMS and ICP-AES.

At the technical meeting, it was recalled that strict observance of the extraction protocol would be a criterion for considering the results for discussion. The participants recommended that a tolerance of $\pm 30\%$ be included in the extraction protocol for the shaker speed. Most of the errors detected were rather due to e.g. calibration errors than to the application of the extraction procedure. A summary of the statistical evaluation is given in the certification report [25]. The certified values and their uncertainties are given in Tables 9.7a–c as mass fractions of the respective extracts obtained at the first, second and third steps in mg kg^{-1} (based on dry mass).

9.5.5. Participating laboratories

The CRM was collected and prepared by the Joint Research Centre, Environment Institute in Ispra (Italy); the homogeneity and stability studies were carried out by the Universidad de Barcelona, Departamento de Química Analítica (Spain). The following laboratories participated in the certification campaign: Agriculture and Food Development Authority, Wexford (Ireland); Agricultural Research Centre, Institute for Crops and Soil, Jokioinen (Finland); Bundesanstalt für Materialforschung und Prüfung, Berlin (Germany); Chalmers University of Technology, Göteborg (Sweden); Estação Agronómica Nacional, Oeiras (Portugal); Estación Experimental del Zaidín, C.S.I.C., Granada (Spain); Institut National d'Agronomie, Laboratoire de Chimie Analytique, Paris (France); Institut National de Recherche Agronomique, Arras (France); Joint Research Centre, Environment Institute, Ispra (Italy); Laboratoire Central des Ponts et Chaussées, Division Eau, Bouguenais (France); The Macaulay Land Use Research Institute, Aberdeen (United Kingdom); Universidad de Barcelona, Dept. de Química Analítica, Barcelona (Spain); Universidade Nova de Lisboa, Monte da Caparica (Portugal); Universiteit Gent, Laboratory of Analytical and Agro-Chemistry, Ghent (Belgium); University of Strathclyde, Department of Pure and Applied Chemistry, Glasgow (United Kingdom); University of Reading, Department of Soil Sciences, Reading (United Kingdom)

TABLE 9.7A

CERTIFIED CONTENTS OF EXTRACTABLE CONTENTS OF Cd, Cr, Cu, Ni, Pb AND Zn — FIRST STEP

	Certified value (mg kg^{-1})	Uncertainty (mg kg^{-1})	p
Cd	4.14	0.23	11
Cr	0.36	0.04	12
Cu	8.32	0.46	9
Ni	8.01	0.73	10
Pb	2.68	0.35	11
Zn	264	5	12

TABLE 9.7B

CERTIFIED CONTENTS OF EXTRACTABLE CONTENTS OF Cd, Cr, Cu, Ni, Pb AND Zn — SECOND STEP

	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	p
Cd	3.08	0.17	10
Ni	6.05	1.09	11
Pb	33.1	10.0	9
Zn	182	11	12

TABLE 9.7C

CERTIFIED CONTENTS OF EXTRACTABLE CONTENTS OF Cd, Cr, Cu, Ni, Pb AND Zn — THIRD STEP

	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	p
Cd	1.83	0.20	11
Ni	8.55	1.04	9
Pb	109	13	12

p: number of data sets

9.6. PCBS IN FRESHWATER SEDIMENT

9.6.1. Introduction

Chlorobiphenyls (CBs) have been used as transformer oils, dielectric fluids, hydraulic fluids and flame retardants. Due to their chemical stability and their lipophilic character, CBs persist in the environment and accumulate in organisms, especially in top predators of the food chain. CBs are included in the black list of EEC directive 76/464/EEC. Although their use is severely restricted, the large quantities still being discharged and their low mobility and persistence leave CBs as a serious environmental problem in the years to come. CBs are frequently determined in freshwater sediments in the framework of research, monitoring programmes and environmental legislation. Arguments about quality of the data may seriously affect international negotiations, e.g. the International Rhine Commission, the guidelines of the Oslo Convention with respect to dumping of dredged material at sea; this implies that CRMs should be available for QC purpose and a freshwater CRM has hence be prepared. The CB contents in CRM 536 are neither extremely low nor extremely high; the sediment characteristics such as organic matter content can be regarded as being representative of many European freshwater sediments. The certified CBs have been chosen on the basis of their occurrence in the environment,

their toxicity and for specific aspects of their determinations by chromatographic techniques.

9.6.2. Production of the candidate reference material

The sediment material of interest had to show a CB content sufficient to be determined with an acceptable precision and not reflecting heavy pollution such as harbour dredges. Based on records of the Dutch National Institute for Water Management and Waste Water Treatment (RIZA) on the concentrations of pollutants in the sediments of the big rivers, a sampling location was selected where the sediment was expected to meet the requirements mentioned above. A total of 1500 kg of wet material was taken from a sediment top layer of about 70 cm.

A pilot study on the heat sterilisation process of the sediment material was performed to evaluate the losses of CBs. From the results it was concluded that the heat treatment has to be carried out at ca. 120°C over a period of 2 h in order to have the least effect on the CB content. Immediately after sampling, the sediment was air-dried at 40°C over a period of several weeks with continuous churning up and removal of larger objects. The dried sediment was then sieved (pore size 2 mm) and jet-milled for a particle size of less than 125 µm and heat sterilised at 120°C for 2 h, homogenised in a 250 L multi-purpose mixer during semi-automatic filling of bottles. The material was finally packed in 4400 bottles, each one containing 40 g.

The within- and between-bottle homogeneity was verified and the variances were compared with the method variance (assessed on the basis of replicate analyses of an extract). A 500 mg portion of the sediment was extracted in a Soxhlet apparatus with 120 mL of a mixture of n-hexane and acetone 90/10 (v/v) for 6 h. The extract was concentrated and taken into a volume of 1 mL iso-octane. The concentrated extract was eluted over 2 g deactivated silica (with 15% water) with 15 mL of petroleum ether. The eluate was concentrated to a volume of 1 mL and treated with 1 mL of a solution containing 34 g L⁻¹ tetrabutylammonium sulphate, and sodium sulphite and isopropanol for the removal of sulphur. The concentrated eluate was cleaned over a column packed with 1.5 g deactivated silica (with 5% water). An internal standard solution (CB 198) was added to the clean extract. The mixture was separated on an Ultra-2 column (length 50 m, internal diameter 0.2 mm, film thickness 0.33 mm, He carrier gas, 65 min gradient from 90 to 275°C) with electron capture detection. No significant difference in the variance of the between- and the within-bottle series and the analytical method itself. Therefore, it was concluded that the material is homogeneous for CBs.

The stability was tested over a period of 12 months at -20°C, +20°C and +40°C. All CB compounds were found to be stable at +20°C. For storage at +40°C, however, a considerable number of significantly lower values occurred: for all congeners after 1 and after 6 months storage and for the lowest chlorinated congener (CB 28) after 12 months storage. Although the low values for 1 and 6 months storage were not confirmed by low values for 3 and 12 months, the fact that the decline is more pronounced for the lower chlorinated congeners may indicate a potential instability of these compounds at +40°C. Previous experience with similar certified reference materials of BCR (e.g.

CRM 481, CBs in soil) has demonstrated a good stability of the CBs. As a consequence, storage of the sediment at a maximum of +20°C is recommended.

9.6.3. Certification

The final determination of the CBs was performed by capillary gas chromatography using either mass selective detection or electron capture detection. Each participant had validated its method by performing experiments on recovery, extraction efficiency, procedure blanks and detector linearity.

Participants were requested to use as much as possible the CRM calibrants from BCR [26]; if CRMs were not available, alternative calibrants of high and, if possible, certified purity were used. The use of at least one internal standard was mandatory; the participants, however, were left free to select the internal standard(s) best suited to their methods. They had to ensure that the selected internal standard(s) did not occur in the candidate reference material or did not interfere with compounds present in the material.

Details on the methods used (extraction, clean-up, separation) are given in the certification report [26]. Extraction was either carried out ultrasonically or by Soxhlet using organic solvent, e.g. hexane, acetone, n-pentane, dichloromethane. Clean-up was performed e.g. by treatment with concentrated H_2SO_4 followed by desulphurisation, column chromatography with e.g. activated alumina, silica etc. Capillary gas chromatography was used, identifying the CB compounds on the basis of their relative retention times and, in case of mass selective detection, on the basis of their ion masses. In all cases, at least two columns of different polarity have been used for quantification. For each CB, the participant selected the best suited column.

Three participants used mass selective detection (MSD), the others electron capture detection (ECD). In case of MSD, the appropriate masses were monitored for the CBs and the internal standards. Quantification was either by peak area or by peak height.

The recoveries of the methods were determined by spiking the sediment with known levels of each of the CBs. Spiking was either on a single level in triplicate or on three levels in at least singlefold. In case of recovery values over 100% no corrections have been on the final CB contents in the sediment. In case of recovery values under 100% corrections have been made. Data for CBs with recoveries significantly differing from 100% were discussed and possibly rejected. When isotope dilution with labelled CBs was applied, results were not corrected for recovery (isotope ratio calculation principle). Recoveries ranged from ca. 70 to 110%.

The quality control procedures, recovery experiments and blank values were examined. It was decided to reject all data obtained with the following recovery values:

- less than 70%,
- having a standard deviation over 15%,
- exceeding 100% by more than the reported standard deviation.

A number of data were withdrawn, the reasons including insufficient chromatographic separation, extreme large or bimodal distribution of the five replicate measurements made by participants and calibration errors.

The certified values along with their uncertainties are given in Table 9.8.

TABLE 9.8

CERTIFIED VALUES FOR CBs IN FRESHWATER SEDIMENT, CRM 536

Compound (IUPAC number)	Certified value ($\mu\text{g kg}^{-1}$)	Uncertainty ($\mu\text{g kg}^{-1}$)	No of accepted sets of results
CB 28	44	5	10
CB 52	38	4	14
CB 101	44	4	15
CB 105	3.5	0.6	8
CB 118	28	3	16
CB 128	5.4	1.2	8
CB 138	27	4	6
CB 149	49	4	13
CB 153	50	4	14
CB 156	3.0	0.4	8
CB 163	17	3	6
CB 170	13.4	1.4	7
CB 180	22	2	14
CB 28	2,4,4'-trichlorobiphenyl		
CB 52	2,5,2',5'-tetrachlorobiphenyl		
CB 101	2,4,5,2',5'-pentachlorobiphenyl		
CB 105	2,3,4,3',4'-pentachlorobiphenyl		
CB 118	2,4,5,3',4'-pentachlorobiphenyl		
CB 128	2,3,4,2',3',4'-hexachlorobiphenyl		
CB 138	2,3,4,2',4',5'-hexachlorobiphenyl		
CB 149	2,4,5,2',3',6'-hexachlorobiphenyl		
CB 153	2,4,5,2',4',5'-hexachlorobiphenyl		
CB 156	2,3,4,5,3',4'-hexachlorobiphenyl		
CB 163	2,3,5,6,3',4'-hexachlorobiphenyl		
CB 170	2,3,4,5,2',3',4'-heptachlorobiphenyl		
CB 180	2,3,4,5,2',4',5'-heptachlorobiphenyl		

9.6.4. Participating laboratories

The preparation of the material has been performed by the Instituut voor Milieuvraagstukken, Vrije Universiteit Amsterdam (The Netherlands), the Vakgroep Bodemkunde en Plantevoeding, Landbouwniversiteit Wageningen (The Netherlands), and the Institute for Reference Materials Measurements in Geel (Belgium). The homogeneity and stability studies were carried out by the Vrije Universiteit Amsterdam (The Netherlands). The following laboratories participated in the certification: Bureau de Recherches Géologiques et Minières, Orléans (France); Ecole Polytechnique Fédérale de Lausanne, Lausanne (Switzerland); Eidgenössische Materialprüfungs- und Forschungsanstalt, Dübendorf (Germany); Elf Atochem S.A., Service Analyse Environnement, Levallois (France); CETS, Institut Químic de Sarrià, Barcelona (Spain); CSIC-CID, Barcelona (Spain); Direcção Geral do Ambiente, Lisboa (Portugal); Instituut

voor Milieuvraagstukken, Vrije Universiteit Amsterdam (The Netherlands); Institutet för Tillämpad Miljöforskning, Stockholms Universitet, Stockholm (Sweden); Milieu- en Toxicologische Chemie, Universiteit van Amsterdam (The Netherlands); République et Canton de Genève, Services du Chimiste Cantonal, Genève (Switzerland); Rijksinstituut voor Visserijonderzoek, IJmuiden (The Netherlands); The Scottish Office, Agriculture and Fisheries Department, Marine Laboratory, Aberdeen (United Kingdom); Vlaamse Instelling voor Technologisch Onderzoek, Mol (Belgium); Ville de Rouen, Laboratoire Municipal et Régional, Rouen (France).

9.7. PAHs IN FRESHWATER SEDIMENT

9.7.1. Introduction

Many Polycyclic Aromatic Hydrocarbons (PAHs) possess carcinogenic and mutagenic activities or are toxic for aquatic organisms. Although PAHs are formed by natural processes, such as forest fires and coalification, their present origin is mainly anthropogenic: incomplete combustion of both fossil fuels and wood and the application of PAH-containing products in wood conservation, ship hull protection and aluminum production. PAHs are frequently determined in freshwater sediments in the framework of research, monitoring programmes and environmental legislation. Accurate information about PAHs in freshwater sediments is required for environmental management which implies that CRMs should be available for QC purpose and a freshwater CRM has hence be prepared. The PAH contents in CRM 535 are neither extremely low nor extremely high; the sediment characteristics such as organic matter content can be regarded as being representative of many European freshwater sediments. The certified PAHs have been chosen on the basis of their occurrence in the environment and their biologic activities.

9.7.2. Production of the candidate reference material

The sediment material of interest had to show a PAH content sufficient to be determined with an acceptable precision and not reflecting heavy pollution such as harbour dredges. Based on records of the Dutch National Institute for Water Management and Waste Water Treatment (RIZA) on the concentrations of pollutants in the sediments of the big rivers, a sampling location was selected where the sediment was expected to meet the requirements mentioned above. A total of 1500 kg of wet material was taken from a sediment top layer of about 70 cm.

A pilot study on the heat sterilisation process of the sediment material was performed to evaluate the losses of PAHs. Heating of the sediment at 105°C for 70 h led to losses of up to 60% of some PAHs. From the results it was concluded that the heat treatment has to be carried out at ca. 120°C over a period of 2 h in order to have the least effect on the PAH content. Immediately after sampling, the sediment was air-dried at 40°C over a period of several weeks with continuous churning up and removal of larger objects. The dried sediment was then sieved (pore size 2 mm) and jet-milled for a particle

size of less than 125 μm and heat sterilised at 120°C for 2 h, homogenised in a 250 L multi-purpose mixer during semi-automatic filling of bottles. The material was finally packed in 4600 bottles, each one containing 40 g.

The within- and between-bottle homogeneity was verified and the variances were compared with the method variance (assessed on the basis of replicate analyses of an extract). A 500 mg portion of the sediment was extracted in a Soxhlet apparatus with 120 mL of a mixture of n-hexane and acetone 90/10 (v/v) for 6 h. The extract was concentrated and taken into a volume of 1 mL iso-octane. The concentrated extract was eluted over 1.5 g fully activated silica with 15 mL of a 85/15 (v/v) n-pentane/dichloromethane mixture. A first fraction of 7 mL pure n-pentane was discarded. The eluate was concentrated and taken into a volume of 1 mL of acetonitrile. The concentrate was separated by HPLC with a C-18 column (length 25 cm, internal diameter 4.6 mm, particle size 5 mm), with an acetonitrile/water gradient (45/55 to 100/0 (v/v)) associated to a fluorescence and a diode-array absorption detection. No significant difference in the variance of the between- and the within-bottle series and the analytical method itself. Therefore, it was concluded that the material is homogeneous for PAHs.

The stability was tested over a period of 12 months at -20°C, +20°C and +40°C. All PAH compounds were found to be stable at +20°C. At +40°C, instability was noticed after 6 months for almost all PAHs. As a consequence, storage of the sediment over long periods is recommended at +4°C.

9.7.3. Certification

The final determination of the PAHs was performed by capillary gas chromatography using flame ionisation or mass selective detection, or by high-performance liquid chromatography using fluorescence or absorption detection. Each participant had validated its method by performing experiments on recovery, extraction efficiency, procedure blanks and detector linearity.

Participants were requested to use as much as possible the CRM calibrants from BCR [25]; if CRMs were not available, alternative calibrants of high and, if possible, certified purity were used. The use of at least one internal standard was mandatory; the participants, however, were left free to select the internal standard(s) best suited to their methods. They had to that the selected internal standard(s) did not occur in the candidate reference material or did not interfere with compounds present in the material.

Details on the methods used (extraction, clean-up, separation) are given in the certification report [27]. Extraction was either carried out ultrasonically or by Soxhlet using organic solvent, e.g. cyclohexane, acetone, methanol, toluene. Clean-up was performed with alumina cartridge or silica with an elution with e.g. cyclohexane, toluene, n-pentane etc. Capillary gas chromatography was used, identifying the PAH compounds on the basis of their relative retention times and, in case of mass selective detection, on the basis of their ion masses. In all cases, at least two columns of different polarity have been used for quantification. For each PAH, the participant selected the best suited column.

Seven participants used mass selective detection (MSD), two used flame ionisation detection (FID). In case of MSD, the appropriate masses were monitored for the PAHs

and the internal standards. Quantification was either by peak area or by peak height. Five participants used HPLC with reversed phase columns and gradient elution with different types of mobile phases or different column temperatures in order to have two systems of different selectivity.

All participants used fluorescence detection with programmable excitation and emission wavelengths. Three of them used diode-array absorption detection in addition: depending on the PAH, the signal from either the fluorescence or the absorption detector was selected.

The recoveries of the methods were determined by spiking the sediment with known levels of each of the PAHs. Spiking was either on a single level in triplicate or on three levels in at least singlefold. In case of recovery values over 100% no corrections have been on the final PAH contents in the sediment. In case of recovery values under 100%, corrections have been made. Data for PAHs with recoveries significantly differing from 100% were discussed and possibly rejected. When isotope dilution with labelled PAHs was applied, results were not corrected for recovery. Recoveries ranged from ca. 75 to 105%.

The quality control procedures, recovery experiments and blank values were examined. It was decided to reject all data obtained with recovery values exceeding 100% by more than the standard deviation. Some of the data were withdrawn, the reasons including bad peak shapes, doubts about calibration, insufficient chromatographic separation, doubts about peak purity incorrect peak assignment.

The certified values along with their uncertainties are given in Table 9.9.

9.7.4. Participating laboratories

The preparation of the material has been performed by the Instituut voor Milieuvraagstukken, Vrije Universiteit Amsterdam (The Netherlands), the Vakgroep Bodemkunde en Plantenvoeding, Landbouwniversiteit Wageningen (The Netherlands), and the Institute for Reference Materials Measurements in Geel (Belgium). The homogeneity and stability studies were carried out by the Vrije Universiteit Amsterdam (The Netherlands). The following laboratories participated in the certification:

TABLE 9.9

CERTIFIED VALUES FOR PAHs IN FRESHWATER SEDIMENT, CRM 535

Compound	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	No of accepted sets of results
Pyrene	2.52	0.18	12
Benz(a)anthracene	1.54	0.10	13
Benzo(a)pyrene	1.16	0.10	11
Benzo(e)-pyrene	1.86	0.13	13
Benzo(b)fluoranthene	2.29	0.15	12
Benzo(k)fluoranthene	1.09	0.15	13
Indeno(1,2,3-cd)pyrene	1.56	0.14	14

Biochemisches Institut für Umweltcarcinogene, Grosshansdorf (Germany); Centre d'Analyse et de Recherche sur les Substances Organiques, Vernaison (France); Institut Universitaire Romand de Santé au Travail, Lausanne (Switzerland); Institut de Recherches Hydrologiques, Vandoeuvre (France); Instituut voor Milieuvraagstukken, Vrije Universiteit Amsterdam (The Netherlands); Istituto Nazionale per la Ricerca sul Cancro, Genova (Italy); Laboratory of the Government Chemist, Teddington (United Kingdom); Union Technique de l'Automobile, du Motocycle et du Cycle, Monthléry (France); Université de Bordeaux I, Laboratoire de Photophysique et Photochimie Moléculaire, Talence (France); Vlaamse Instelling voor Technologisch Onderzoek, Mol (Belgium); VTT Chemical Technology, Espoo (Finland).

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*Chapter 10***CRMs for soil and sludge analysis****10.1. INTRODUCTION**

Soil and sludge analyses are currently performed by a number of organisations for various purposes, e.g. agronomic studies (plant uptake, soil deficiency etc.) and environmental risk assessment (mobility of contaminants from polluted soils). Determinations of total trace element contents correspond to the vast majority of analyses performed for monitoring purposes (e.g. mapping). Organic compounds (e.g. PCBs, PAHs) are mainly determined for studies of environmental risks. Other types of analyses are performed to study the mobility of inorganic contaminants and their bioavailability on the basis of use of single extraction procedures (e.g. acetic acid, EDTA etc.). Finally, many laboratories performing agricultural analyses frequently determine the aqua-regia soluble fraction of certain elements instead of the total content; information about this parameter is considered to be a reliable estimate of the maximum availability to plants. The determination of the aqua-regia soluble fraction is often determined to estimate the impact of amendment of soils by sewage sludge as requested by national regulations (e.g. in Denmark, Germany, France and The Netherlands).

Materials such as sewage sludges and soils consist of numerous solid phases, all of them differing with respect to their morphological, physical and chemical properties. The geometrical shape of the particles varies from fibres of a few μm diameter but several 100 μm in length, over thin leaf-like particles (kaolinite, mica, etc.) to isometric compact particles such as rounded silica grains and rhombohedral calcite cleavage particles with different mass densities. Owing to the wide variety of matrices encountered, a range of CRMs should be available to verify the analytical quality control of the determinations carried out by the laboratories.

10.2. TRACE ELEMENTS IN SOILS AND SLUDGES**10.2.1. Introduction**

Soil reference materials certified for their trace element contents have been produced by the BCR at the beginning of the 1980's, namely calcareous soil (CRM 141) and light sandy soil (CRM 142) [1]. Due to their success, supplies were rapidly exhausted and it was decided to replace them by the CRMs 141R and 142R [2–5]. Sewage sludge amended soils were prepared for completing this series, namely CRMs 143R and 144R.

Sludge CRMs were also considered as reference materials needed for QC purposes

and three types of materials were produced, namely, sewage sludge of a domestic origin (CRM 146), sewage sludge (CRM 145) and sewage sludge of mainly industrial origin (CRM 146) [7].

10.2.2. Production of the reference materials

Calcareous loam soil, CRM 141R: Two hundred forty kg of soil was collected by shovelling from the surface to a depth of 10 cm (collection of top layers). Stones, plant litter or wood pieces were removed manually. The material was air dried in thin layers and continuously moved and mixed to avoid fungi development. At a residual moisture content of 3.7% (determined by drying 1 g to constant mass at 105°C in an oven), the material was passed through a 2 mm sieve discarding the fraction > 2 mm and eliminating extraneous particles such as stones, roots, fragments of plastic and metal. The further processing consisted of crushing with a Retsch SR3 mill with an inner 0.75 mm sieve. After crushing the material was further sieved mechanically to obtain the fraction < 90 µm. This material was inserted in a rigid mixing drum with out-of axis-fins. The drum was kept rotating for 170 h at 40 rpm until a sufficiently homogeneous material with particle size < 90 µm was obtained. The remaining material available for bottling was about 60 kg. It was bottled in 50 g portions in clean brown glass bottles closed with a plastic insert and screw cap.

Light sandy soil, CRM 142R: The sandy soil was collected by shovelling from the surface to a depth of ca. 10 cm, avoiding stones, plant litter or wood pieces. The material was air-dried and heat sterilised, followed by sieving, and discarding the fraction > 2 mm. The subsequent processing consisted of grinding in a hammer with large openings (about 2 mm), sieving (<90 µm) and blending of the fine fraction for 10 days in a special blending drum until a sufficiently homogeneous material was obtained. The material was bottled in 50 g portions in clean brown glass bottles provided with a PTFE ball for re-homogenisation and closed with a plastic insert and screw cap.

Sewage-sludge amended soil, CRM 143R: A convenient sewage sludge-amended soil showing sufficient similarity to the CRM 143 was found at the Agricultural Research Station in Hagen (Germany). Portions of sewage sludge were added to the soil for approximately 10 years with the aim of field scale carry-over experiments of metals to crops. With regard to critical quantities such as Al_2O_3 , Fe_2O_3 and TiO_2 content, the new soil compares well with the matrix composition of the exhausted reference material. The materials were collected by shovelling from the surface to a depth of ca. 10 cm, avoiding stones, plant litters or wood pieces. The CRM 143R was air-dried and heat-sterilised, followed by sieving, and discarding the fraction >2 mm. The subsequent processing consisted of grinding in a hammer mill with large openings (about 2 mm), sieving (<90 µm) and blending of the fine fraction for 10 days in a special blending drum until a sufficiently homogeneous material was obtained. The material was bottled in 50 g portions in clean brown glass bottles provided with a PTFE ball for re-homogenisation and closed with a plastic insert and screw cap.

Sewage sludge from domestic origin, CRM 144R: An adequate sewage sludge was collected at the Rovereto waste water purification plant near Trento, (Italy). The mainly civil dwellings guaranteed a relatively low metal concentration. A preliminary analysis

of the sludge revealed that the heavy metal concentrations are lower than the concentrations in the original sludge CRM thus reflecting the present wastewater conditions as a consequence of the pollution measures taken. Several hundred kilograms of sludge material were collected from a drying oven at the wastewater treatment plant (temperature of 175–180°C). Through the material looked rather solid, it was found to contain 3.3% of water (determined by drying 1 g of material to constant mass at 105°C in an oven). The material was passed through a 2 mm sieve discarding the fraction > 2 mm and eliminating extraneous particles such as stones, roots, fragments of plastic and metal. The fraction < 2 mm was divided into 10 batches of 10 kg which all underwent gamma irradiation at a dose of 25 kGy (sufficient to sterilise the materials). The batches were pooled together for further processing which consisted of crushing with a Retsch SR3 mill with an inner 0.75 mm sieve. After crushing the material was further sieved mechanically to obtain the fraction < 90 µm. This material was inserted in a rigid mixing drum with out-of axis-fins. The drum was kept rotating for 170 h at 40 rpm until a sufficiently homogeneous material with particle size < 90 µm was obtained; the optimal homogenisation time was based on previous experience with this type of material. The final material was bottled in 50 g portions in clean brown glass bottles closed with a plastic insert and screw cap; the bottling procedure was carried out in a stepwise manner: twenty bottles were filled while the drum was stationary, then the drum was operated again for 3 minutes and 20 other bottles were filled etc.

Sewage sludge, CRM 145R: A sewage sludge with a matrix composition that compares well with that of the former CRM 145 was collected in Italy. The material was sampled at a water purification plant; it was taken from a concrete basin where it was dumped following filtration. The moisture content was found to be 80%. Anything extraneous to the sludge, e.g. pieces of plastic, aluminium or wood, were removed manually. The sludge material was air-dried, lumps were pulverised and the material was passed through a 2 mm sieve, discarding the fraction > 2 mm. The material underwent γ-ray irradiation at a dose of 25 kGy. The subsequent processing consisted of grinding in a hammer mill with large openings (about 2 mm), sieving (<90 µm) and blending of the fine fraction for 10 days in a special blending drum until a sufficiently homogeneous material was obtained. The material was bottled in 40 g portions in clean brown glass bottles provided with a PTFE ball for re-homogenisation and closed with a plastic insert and screw cap.

Sewage sludge from industrial origin, CRM 146R: A sewage sludge with an interesting high content of metals under regulation was collected in Maison Lafitte in the region of Paris, (France). A preliminary analysis of the sludge revealed that the heavy metal concentrations are lower than in the original sludge CRM thus reflecting the present wastewater conditions as a consequence of the pollution measures taken. The matrix composition corresponds quite well with that of the original sludge CRM considering the natural variability of sludges. 250 kg of sludge containing about 50% of solid material was collected. The sludge was air dried in thin layers and continuously moved and mixed to avoid fungi development. The material was passed through a 2 mm sieve discarding the fraction > 2 mm and eliminating extraneous particles such as stones, roots, fragments of plastic and metal. The fraction < 2 mm was divided into 10 batches of 10 kg which all underwent gamma irradiation at a dose of 25 KGy (sufficient to sterilise the material). The batches were pooled together for further processing which

consisted of crushing with a Retsch SR3 mill with an inner 0.75 mm sieve. After crushing, the material was further sieved mechanically to obtain the fraction $< 90 \mu\text{m}$. This material was inserted in a rigid mixing drum with out-of axis-fins. The drum was kept rotating for 170 h at 40 rpm until a sufficiently homogeneous material with particle size $< 90 \mu\text{m}$ was obtained; the optimal homogenisation time was based on previous experience with this type of material. The final material was bottled in 50 g portions in clean brown glass bottles closed with a plastic insert and screw cap; the bottling procedure was carried out in a stepwise manner: twenty bottles were filled while the drum was stationary, then the drum was operated again for 3 minutes and 20 other bottles were filled; the operation was repeated until the whole material was bottled.

Sewage sludge, CRM 597: The sewage sludge material (CRM 597) has been produced on purpose for the certification of chromium. Typically sewage sludge composition reflects the industrial activities in the catchment area of the sewage sludge plant which might be dominated by a single chromium source. In order to produce a candidate reference material which represents a broader spectrum of sources and hence, a more diversified of chromium species and associations with different solid phases. A blend material has been produced, made up from 3 different materials. The sewage sludge was taken from different sewage plants (Mezzocorona and Rovereto, Italy and Paris, France). The blend material was air-dried at ambient temperature, further dried at 105°C after crushing the lumps and large aggregates, and passed through a large Retsch hammer mill, equipped with tungsten carbide tools and a circular sieve with 2 mm aperture. The obtained powder was passed over a 125 μm sieve and the fraction $> 125 \mu\text{m}$ discarded. The fraction $< 125 \mu\text{m}$ was homogenised for 2 weeks in a mixing drum. In order to test the completeness of mixing, 10 sub-samples were taken at different positions in the mixing drum for bulk homogeneity testing. Each bottle contains ca. 40 g.

Keeping in mind that all the different phases show distinct trace element concentrations, sometimes differing over orders of magnitude, it is likely that any segregation of particles, for example through vibrations during transport could lead to considerable inhomogeneities with respect to the trace elements. Re-homogenisation prior to sub-sampling is therefore mandatory.

The homogeneity of the soil and sludge materials was verified using the usual BCR procedure (within- and between-bottle homogeneity assessment). In the case of CRMs 141R, 144R and 146R, the homogeneity study was performed for the elements Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, S, Sn, V and Zn at levels of intakes of 50, 100 and 250 mg, using simultaneous ICP-AES after pressurised wet ashing with HNO_3 ; a similar study was carried out using an aqua-regia digestion (following the DIN standard). In the case of CRMs 142R, 143R and 145R, the homogeneity was verified for the elements Ba, Ca, Cr, Cu, Fe, Mg, Mn, Ni, P, Sr, Ti, V and Zn at levels of intake of 100 and 250 mg, using the same technique as for CRM 141R. For the CRMs 141R, 144R and 146R, the homogeneity was found to be satisfactory at sample intakes as low as 50 g (for total element contents) [5]. The somewhat higher CVs for the 250 mg sample intake for aqua-regia soluble contents were a consequence of the digestion method used; the CRM is considered to be homogeneous at a minimum sample intake of 250 mg and of course at 3 g level as stipulated in the

DIN standard [5]. In the case of the CRMs 142R, 143R and 145R, the homogeneity was demonstrated at the 250 mg level of intake [3].

10.2.3. Certification

Table 10.1 gives an overview of the techniques used by the participating laboratories for the different elements. The pretreatment techniques were digestion with a combination of acids in a pressurised or atmospheric mode under reflux, programmed dry ashing or combustion. The use of HF was mandatory for complete digestion. Matrix matching of the calibrants (addition of e.g. Ca, P or Fe) was necessary when applying an external calibration curve with spectrometric techniques as method of final determination. A more detailed description of the techniques is given in the certification reports [3,5].

For the certification of aqua-regia soluble trace element contents, it was mandatory that the participants follow strictly the same extraction procedure. The digestion method used was the DIN standard (German Norm 38414-S7) which is based on the digestion of 3 g material with HCl/HNO₃ following a detailed protocol that is described elsewhere [3,5].

A generally good agreement was obtained for all elements in the CRMs 141R, 144R and 146R, which enabled all total and aqua-regia contents to be certified. With respect to CRM 142R, the total Cr and Zn contents and the aqua-regia soluble contents of Co, Cr, Cu and Mn could not be certified, owing to a high a spread of results and explained sources of systematic Errors. For CRM 143R, it was decided that the total content of Cr and the aqua regia soluble Co, Cu and Hg content could not be certified. The total Cr content and the aqua regia soluble Cd, Co, Mn and Hg content could not be certified in CRM 145R; the mean values could, however, be considered as good indicative values. The certified values are given in Tables 10.2–4.

With respect to CRM 597, a tendency of the results of the non-destructive determinations to be higher than the majority of the results obtained with destructive methods

TABLE 10.1

SUMMARY OF THE TECHNIQUES OF FINAL DETERMINATION FOR TRACE ELEMENTS IN SOILS AND SEWAGE SLUDGES

Element	Techniques
Cd	EDXRF, ETAAS, FAAS, ICP-AES, ICPMS, IDMS
Co	FAAS, ICP-AES, ICPMS, INAA
Cr	DCP-AES, ETAAS, FAAS, ICP-AES, ICPMS, IDMS, INAA, RNAA
Cu	DCP-AES, EDXRF, FAAS, ICP-AES, ICPMS, IDMS
Hg	CVAAS, CVAFS, HGAAS, ICPMS, RNAA
Pb	DCP-AES, ETAAS, FAAS, ICP-AES, ICPMS, IDMS
Mn	DCP-AES, EDXRF, FAAS, ICP-AES, ICPMS, INAA
Ni	DCP-AES, ETAAS, FAAS, ICP-AES, ICPMS, IDMS
Zn	DCP-AES, EDXRF, FAAS, ICP-AES, ICPMS, IDMS, INAA, RNAA

TABLE 10.2

CERTIFIED VALUES IN CRMS 141R AND 142R (IN mg kg⁻¹)

Element	CRM 141R	CRM 142R
Total content		
Cd	14.6 ± 0.5	0.34 ± 0.04
Co	10.5 ± 0.4	12.1 ± 0.7
Cr	195 ± 7	n.c.
Cu	46.4 ± 1.8	69.7 ± 1.3
Pb	57.2 ± 1.2	40.2 ± 1.9
Mn	683 ± 16	970 ± 16
Hg	0.25 ± 0.02	0.067 ± 0.011
Ni	103 ± 3	64.5 ± 2.5
Zn	283 ± 5	n.c.
Aqua regia		
Cd	14.0 ± 0.4	0.25 ± 0.01
Co	9.2 ± 0.5	n.c.
Cr	138 ± 5	n.c.
Cu	46.9 ± 1.8	n.c.
Pb	51.3 ± 2.0	25.7 ± 1.6
Mn	653 ± 16	n.c.
Hg	0.24 ± 0.03	n.c.
Ni	94 ± 5	61.1 ± 1.5
Zn	270 ± 8	93.3 ± 2.7

n.c. not certified

was noticed. Since this difference was not significant, it had no consequences for the certification of chromium, which was certified with a value of (203 ± 6) mg kg⁻¹.

10.2.4. Participating laboratories

The preparation of the materials was performed at the Joint Research Centre, Environment Institute, of Ispra (Italy), whereas the homogeneity and stability studies were carried out by the GSF-Forschungszentrum für Umwelt und Gesundheit in Oberschleißheim (Germany) and the Energieonderzoek Centrum Nederland in Petten (The Netherlands), respectively. The analyses were performed by the following laboratories: Agricultural Research Centre of Finland, Jokionen (Finland); Agriculture and Food Development Authority, Wexford (Ireland); CNRS, Service Central d'Analyse, Vernaison (France); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Ecole Européenne des Hautes Etudes des Industries Chimiques, Strasbourg (France); GSF-Forschungszentrum für Umwelt und Gesundheit, Oberschleißheim (Germany); INRA, Station d'Agronomie, Villenave d'Ornon (France); Institute for Soil Fertility, Haren (The Netherlands); Institute für Analytische Chemie, Technische Universität

TABLE 10.3

CERTIFIED VALUES IN CRMS 143R AND 144R (IN mg kg⁻¹)

Element	CRM 143R	CRM 144R
Total content		
Cd	71.8 ± 1.2	1.82 ± 0.10
Co	12.3 ± 0.3	15.0 ± 0.6
Cr	n.c.	104 ± 3
Cu	130.6 ± 1.4	308 ± 7
Pb	179.7 ± 2.1	106 ± 4
Mn	904 ± 13	208 ± 3
Hg	1.10 ± 0.07	3.14 ± 0.23
Ni	299 ± 5	47.7 ± 1.1
Zn	1055 ± 14	932 ± 23
Aqua regia		
Cd	72.0 ± 1.8	1.84 ± 0.07
Co	n.c.	13.3 ± 0.5
Cr	426 ± 12	90 ± 6
Cu	n.c.	300 ± 11
Pb	174 ± 5	96.0 ± 1.5
Mn	858 ± 11	189 ± 6
Hg	n.c.	3.11 ± 0.18
Ni	296 ± 4	44.9 ± 1.5
Zn	1063 ± 16	919 ± 16

n.c. not certified

Wien (Austria); Macaulay Institute for Soil Research, Aberdeen (United Kingdom); Ministère des Affaires Economiques, Brussels (Belgium); Risø National Laboratory, Roskilde (Denmark); Universidad de Barcelona, Departamento de Química Analítica (Spain); Università di Pavia, Dipartimento di Chimica Nucleare (Italy); Universiteit Gent, Instituut voor Nucleaire Wetenschappen, Ghent (Belgium).

10.3. EXTRACTABLE TRACE ELEMENTS IN SOILS

10.3.1. Introduction

The eco-toxicity and mobility of metals in the environment depends strongly on their specific chemical forms or types of binding rather than the total element contents. Consequently these have to be determined in order to assess the toxic effects and geochemical pathways. The determination of specific chemical species or binding forms is difficult and often hardly possible. Therefore, in practice determinations of broader 'operationally or functionally defined' forms or phases can be a reasonable compromise;

TABLE 10.4

CERTIFIED VALUES IN CRMS 145R AND 146R (IN mg kg⁻¹)

Element	CRM 145R	CRM 146R
Total content		
Cd	3.50 ± 0.15	18.8 ± 0.5
Co	5.61 ± 0.31	7.39 ± 0.27
Cr	n.c.	196 ± 7
Cu	696 ± 12	838 ± 16
Pb	286 ± 5	609 ± 14
Mn	156 ± 4	324 ± 7
Hg	2.01 ± 0.22	8.62 ± 0.33
Ni	247 ± 7	69.7 ± 4.0
Zn	2122 ± 23	3061 ± 59
Aqua regia		
Cd	n.c.	18.5 ± 0.4
Co	n.c.	6.50 ± 0.31
Cr	307 ± 13	174 ± 7
Cu	707 ± 9	831 ± 16
Pb	282 ± 9	583 ± 17
Mn	n.c.	298 ± 9
Hg	n.c.	8.39 ± 0.25
Ni	251 ± 6	65.0 ± 3.0
Zn	2140 ± 50	3043 ± 58

n.c. not certified

e.g. 'bioavailable' forms of trace elements can give sufficient information to arrive at a sound environmental policy. The increasing concern to assess the bioavailable metal fraction (and thus to estimate the related phytotoxic effects) and the mobility of trace metals upon disposal of e.g. sediment on to a soil (e.g. contamination of ground waters) is reflected by a considerable increase in the frequency of analysis over the last ten years. Single and sequential extraction schemes have been designed in the 80's in order to assess the different retention/release of metals in soil and sediment samples [11–13]. However, the lack of uniformity in the different procedures used did not allow the results to be compared worldwide or the procedures to be validated. The results obtained are defined by the determination of extractable elements using a given procedure; therefore their significance is highly dependent on the extraction protocol performed. Moreover, the lack of suitable reference materials for this type of studies did not enable the quality of the measurements to be controlled. Because of the many pitfalls likely to occur in the use of extraction protocols for soil analysis, the Measurements and Testing Programme has launched a project aiming at harmonising measurements for extractable trace metal contents in soil. This project followed a stepwise approach (through interlaboratory studies) of which the final aim was to certify soil reference

materials for their extractable trace element contents. Two sewage sludge amended soils were prepared and certified for their EDTA- and acetic acid- extractable trace element contents. The project was continued by an interlaboratory study and a subsequent certification of EDTA- and DTPA-extractable trace element contents in a sewage sludge amended calcareous soil (CRM 600). This chapter presents the results of the certification work only; the 'step-by-step' approach for harmonising the extraction schemes is described in more details in chapter 12.

10.3.2. Production of the candidate reference materials

The CRM 483 was collected from Great Billings Sewage farm (Northampton). Some 300 kg of field-moist soil was collected by multiple sampling to a depth of 10 cm and bulked into polyethylene bags for transport to the Macaulay Land Use Research Institute (Aberdeen, UK). The whole soil was air-dried at 30°C for 3 weeks on paper-lined aluminium trays. The dried material was then gently rolled with a wooden roller to break up large aggregates, sieved through a 2 mm round-hole sieve and stored in tightly-sealed polyethylene bags. The soil sample was thoroughly mixed and homogenised by rolling on a clean polyethylene sheet for 3 days with occasional mixing by hand. The whole sample was then gently poured on to a clean polyethelene sheet, mixed, and coned and quartered by hand. The initial sample, nominally 150 kg of air-dry (<2 mm) soil was split by coning and quartering, bulking opposite quarters to form the half samples, and setting one half sample aside. The remaining half sample was again coned and quartered. The coning and quartering procedure continued (6 cycles) until the half-sample weight was approximately 2 kg. From opposite quarters of this half-sample 20 sub-samples were taken alternately by nylon spatula into pre-cleaned brown glass bottles (capped by polyethylene screw caps), each containing approximately 70 g. A total of 1280 bottles were obtained and 128 bottles (2 from each final half-sample) were set aside for homogeneity and stability testing.

The sampling of the CRM 484 (terra rossa soil) was carried out in a farm plot amended with sewage sludge from a water treatment centre located in North-East Catalonia, Spain. Samples were taken from an area of about 250 m² with a small shovel to a depth of about 10 cm and sifted on-site by hand through a 0.5 cm nylon sieve into polyethylene bags. The samples were taken to the Water Treatment Centre and again sieved through a 20 cm diameter nylon sieve with a mesh size of 2 mm into polyethylene bags for transport to the laboratory analytical chemistry of the University of Barcelona. The soil was then spread over a polyethylene sheet and air-dried at 30°C for one week to a final water content of 1.5%. The air-dried soil was packed into a 100 litre polyethylene container, tightly sealed and dispatched to the Environment Institute of the Joint Research Centre of Ispra (Italy) for homogenisation and bottling. The air-dried (<2 mm) soil sample was transferred in total (91 kg) into a mixing drum filled with dry argon and placed on roll-bed capable of handling 100 kg samples. The homogenisation of this soil, with its large spread of particle sizes, from just below 2 mm down to fractions of a micrometer, required particular care. This procedure was, therefore, carried out by mixing in the drum for over 4 weeks. The bottling procedure was performed as follows: to prevent segregation of fine particles, 10 samples were taken from the centre of the

drum immediately upon stopping the rotation of the mixing drum, and were placed into 10 pre-cleaned brown glass bottles, each containing a minimum of 70 g of soil. The drum was again rotated for a further two minutes and a further 10 samples were sub-sampled in the same way into bottles. The sub-sampling and bottling operation was continued until 1000 bottles of the soil were obtained. One hundred bottles, selected sequentially over the whole bottling procedure were sent to the Macaulay Land Use Research Institute for homogeneity and stability testing. Additional details on the characterisation of these materials are found in the certification report [14,15].

The CRM 600 has been collected at San Pellegrino Parmense (Italy), following a prospective study of various sites in Italy which aimed at identifying a material with reasonably high calcium carbonate content. The first 25 cm layer was sampled, following recommendations for soil sampling based on previous experience [16]. Stones and large plant litter were removed prior to sieving at 2 mm mesh. The fraction less than 2 mm was collected in stainless-steel trays in which the material was disposed in thin layers of a few cm of thickness to dry at ambient temperature. The material was sieved again after drying to remove lumps that were formed during the drying process. The residual moisture content at this stage was found to be 3.8% (measured by taking a separate portion of 1 g dried at 105°C until constant mass was attained). The sieved material was transferred into a PVC-mixing drum filled with dry argon, and was homogenised for 12 days at about 48 rpm. The final material was manually bottled in brown glass bottles; the bottling procedure was carried out by filling ten bottles, closing the drum and mixing the material again for 2 minutes before bottling ten other bottles, and so on until only some centimeters of soil remained in the drum (which were discarded). All bottles were closed with an insert and a screw cap and stored at ambient temperature. 1050 bottles each containing about 70 g were produced.

For the homogeneity studies, the extractants (0.05 mol L^{-1} EDTA, 0.43 mol L^{-1} acetic acid and 0.005 mol L^{-1} DTPA) were prepared as laid out in the certification reports [15, 17]. The trace element contents (Cd, Cr, Cu, Ni, Pb and Zn) in the extracts were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) for the CRMs 483/484, flame atomic absorption spectrometry (FAAS) or electrothermal atomic absorption spectrometry with Zeeman background correction (ZETAAS) for the CRM 600. In the case of the CRM 483, little analytical difficulty was experienced as illustrated by the good agreement obtained between the within-bottle and between-bottle CVs; for the CRM 484, lower extractable contents, closer to the detection limits and consequent poorer analytical precision was observed in particular for Cr (EDTA extractable contents), Cd and Pb (acetic acid extractable contents). No particular difficulties were experienced for the CRM 600. On the basis of these results, the materials were considered to be homogeneous at a level of 5 g for EDTA- and acetic acid-extractable contents and 10 g for DTPA-extractable contents (as specified in the extraction protocols).

The stability of the extractable trace element contents was tested at -20 , $+20$ and $+40^\circ\text{C}$ during a period of 12 months and the extractable contents of Cd, Cr, Cu, Ni, Pb and Zn were determined after 1, 3, 6 and 12 months. For the CRMs 483/484, the procedures used were the same as in the homogeneity study, with the exception of Pb for which ETAAS was used as final determination. No instability could be demonstrated and hence the materials were suited as candidate CRMs. In the case of CRM 600, the

material was shown to be stable at +20°C. Some risks of instability were, however, suspected at 40°C due to possible changes in the extractability of some elements (e.g. Cu and Zn); these changes induced by the high storage temperature could be related to changes in the status of the organic matter or in the crystallographic compounds of Fe or Mn. Hence, it is recommended to avoid storage at temperature above 20°C.

10.3.3. Certification

The techniques of final determination were electrothermal atomic absorption spectrometry, flame atomic absorption spectrometry, inductively coupled plasma emission spectrometry, and inductively coupled plasma mass spectrometry.

The choice of the extraction procedures is described in detail elsewhere [18]. On the basis of the results of the first interlaboratory study [19], the choice of EDTA and DTPA for certification was discussed. Whereas EDTA was widely accepted, the choice of DTPA was more criticised because of its operational difficulties; the wide use of the latter would, however, justify the certification of DTPA extractable trace element contents, providing that its limitations in comparison with EDTA were clearly identified.

At the technical meeting, it was recalled that strict observance of the extraction protocols would be a criterion for considering the results for discussion. An example of possible source of discrepancies occurring as the result of non-adherence to the protocols was given by one participating laboratory which used a reciprocating shaker instead of the recommended end-over-end shaker and obtained systematically low results; the repetition of the analyses clearly showed that the error was due to this fact [14,15].

On the basis of this remark, the results of three laboratories using a reciprocating shaker were systematically rejected. The shaker speed was also considered to be an important parameter since it represents one of the most determining factors (along with the shaker type) that conditions the maintenance of the samples in suspension during the extraction. The protocol stipulates a speed of 30 rpm and speeds ranging from 20 to 40 rpm were considered to be acceptable; exceptionally one set of results obtained at 14 rpm were accepted since the soil samples were found to remain in suspension in this particular case. No difference was observed in the final results when filtration, centrifugation or centrifugation followed by filtration was used to separate remaining solid materials from the liquid phase.

For EDTA extractions, a good agreement of results of laboratories using a high speed reciprocating shaking and results of the bulk of the participants (using end-over-end shaking) could be observed; as stressed before, these results could, however, not be retained for certification since the extraction protocol was not strictly followed. For chromium in CRM 483, it was considered that FAAS using air/acetylene flame without a releasing agent was not acceptable [20]. Two laboratories, using nitrous oxide/acetylene flame were on the high side and in the absence of detailed information on this flame in this matrix, these results were rejected. This was also the case for CRM 484 for which a high dispersion of results for chromium did not allow this element to be certified. The mean and standard deviation are given as indicative value. This dispersion was attributed to the operationally-defined procedure and not the detection techniques.

No particular problems were experienced with acetic acid, except for lead in CRM 483 for which some results were too close to detection limits, the data sets of which were consequently removed. As observed for EDTA extracts in CRM 484, the wide dispersion of chromium results could not allow certification; the results are given as indicative value.

In relation to the choice of EDTA or DTPA as extraction procedure for CRM 600, there was a general consensus to prefer EDTA because this procedure is easier to apply (better soil to solution ratio for EDTA). In addition, it was stressed that EDTA and DTPA extractions are closely correlated which renders questionable the use of both extraction procedures at the same time. It was assumed that EDTA extraction enables a complete extraction to be achieved and mimics the mobility of trace metals from soils; DTPA is widely used in the USA and is rather applied to predict plant uptake. The choice of the extractant is, therefore, closely related to the objective of the study.

The certified values and their uncertainties are given in Tables 10.5a–c as mass fractions of the respective extracts, EDTA and DTPA (based on dry mass) in mg kg^{-1} .

10.3.4. Participating laboratories

The sample collection and preparation were performed by the EC Joint Research Centre of Ispra (Italy). The homogeneity and stability of CRMs 483/484 were verified by the Macaulay Land Use Research Institute in Aberdeen (United Kingdom) whereas

TABLE 10.5A

EXTRACTABLE CONTENTS OF Cd, Cr, Cu, Ni, Pb AND Zn IN CRM 483

EDTA	Certified value (mg kg^{-1})	Uncertainty (mg kg^{-1})	p
Cd	24.3	1.3	18
Cr	28.6	2.6	9
Cu	215	11	17
Ni	28.7	1.7	17
Pb	229	8	17
Zn	612	19	17
Acetic acid	Certified value (mg kg^{-1})	Uncertainty (mg kg^{-1})	p
Cd	18.3	0.6	18
Cr	18.7	1.0	17
Cu	33.5	1.6	18
Ni	25.8	1.0	15
Pb	2.10	0.25	12
Zn	620	24	18

p: number of data sets

TABLE 10.5B

EXTRACTABLE CONTENTS OF Cd, Cu, Ni, Pb AND Zn IN CRM 484

EDTA	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	p
Cd	0.51	0.03	14
Cu	88	4	17
Ni	1.39	0.11	15
Pb	47.9	2.6	18
Zn	152	7	17
Acetic acid	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	p
Cd	0.48	0.04	13
Cu	33.9	1.4	18
Ni	1.69	0.15	16
Pb	1.17	0.16	11
Zn	193	7	17

the homogeneity and stability studies for CRM 600 were carried out by the Estación Experimental del Zaidin in Granada (Spain) and the Institut National de Recherche Agronomique in Villenave d'Ornon (France). The analyses for certification were performed by the following laboratories: Agriculture and Food Development Authority, Wexford (Ireland); Agricultural Research Centre, Institute for Crops and Soil, Jokioinen (Finland); Aristotelian University, Laboratory of Analytical Chemistry, Thessaloniki (Greece); Bundesanstalt für Materialforschung und Prüfung, Berlin (Germany); Estación Experimental del Zaidin, Granada (Spain); European Commission, Joint Research Centre, Ispra (Italy); Estação Agronómica Nacional, Departamento de Pedologia, Oeiras (Portugal); Federal Research Centre of Agriculture, Braunschweig-Volkenrode (Germany); Istituto di Chimica Agraria, Università di Bari, Bari (Italy); Institut National d'Agronomie, Paris (France); Institut National de Recherche Agronomique, Villenave d'Ornon (France); Institut National de Recherche Agronomique, Arras (France); Institut für Wasser, Boden und Lufthygiene, Berlin (Germany); Laboratoire Central des Ponts et Chaussées, Bouguenais (France); Landbouw Universiteit, Wageningen (The Netherlands); Metaleurop, Leiter Labor, Arbeit- und Umweltschutz, Nordenham (Germany); Research Institute for Agrobiological Sciences, Haren (The Netherlands); Station Fédérale de Recherches en Chimie Agricole, Liebefeld-Bern (Switzerland); Teknologisk Institut, Kemiteknik, Taastrup (Denmark)); The Macaulay Land Use Research Institute, Aberdeen (United Kingdom); Universidad de Barcelona, Dept. de Química Analítica, Barcelona (Spain); Università di Udine, Dipartimento di Chimica, Udine (Italy); Universiteit Gent, Lab. of Analytical and Agro-Chemistry, Gent (Belgium); University of Strathclyde, Pure and Applied Chemistry, Glasgow (United Kingdom); University of Reading, Dept. of Soil Science, Reading (United Kingdom)

TABLE 10.5C

EXTRACTABLE CONTENTS OF Cd, Cr, Cu, Ni, Pb AND Zn IN CRM 600

EDTA	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	p
Cd	2.68	0.09	18
Cr	0.205	0.022	9
Cu	57.3	2.5	17
Ni	4.52	0.25	17
Pb	59.7	1.8	17
Zn	383	12	17
DTPA	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	p
Cd	1.34	0.04	18
Cr	0.014	0.003	17
Cu	32.3	1.0	18
Ni	3.31	0.13	15
Pb	15.0	0.5	12
Zn	142	6	18

p: number of data sets

10.4. CHLOROBIPHENYLS IN SEWAGE SLUDGE

10.4.1. Introduction

Chlorobiphenyls are determined in sewage sludges to monitor the contamination levels due to release from domestic and industrial waste in the environment. Toxicological studies on the individual CBs and the commercial formulations indicate that these compounds are highly toxic and have both carcinogenic and teratogenic properties in both aquatic and terrestrial animals. Of the possible 209 CBs only about 120 have been detected in environmental compartments. In routine analysis, a selection of these compounds is chosen as indicative of the main groups of CBs found in important matrices. Additional CBs are often determined for research purposes. The particular chlorobiphenyls selected by laboratories in the campaign described on the following basis: presence in industrial mixtures; wide occurrence in environmental samples; toxicity of the CB; scope for identification and determination by chromatographic techniques; mention in legislation.

In conformity with the above criteria, CBs 28, 52, 101, 138, 153 and 180 were originally selected because they occur in many environmental samples and in particular sewage sludge, because they are frequently determined in the EC Member Countries and because they can be separated from the organically rich sludge matrix and from other co-eluting CBs by various analytical techniques. However, in some cases, e.g. CB 28–31, 149–118 and 105–138, the separation of closely eluting pairs on the capillary gas chromatographic

column demands a high degree of analytical skill and instrumental optimisation. But this inbuilt feature of such a reference material does allow the analyst to fully evaluate the method used. In addition to the above mentioned compounds, CB 118 was selected considering that it is the most abundant of the toxic CBs. This section describes the certification of a sewage sludge reference material (CRM 392) for its CB content [21].

10.4.2. Production of the candidate reference material

The material was collected at the sewage plant Geiselbullach in München (Germany), a residential area with some industry. It was obtained through the courtesy of Bayerische Landesanstalt für Bodenkultur und Pflanzenbau (München) where it was freeze-dried. The dry material was transported in well closed polythene bags to the JRC of Ispra for further treatment which included heat sterilisation in a closed container at 130°C for 2–8 h, grinding in a hammer mill using large opening (about 2 mm) for quick through-put and short contact time with the mill itself, sieving at 90 µm, mixing for 10 days in a special mixing drum and bottling in brown glass bottles [22].

The (within- and between-bottle) homogeneity was verified by the determination of the seven selected CBs using the following method: a sample (5 g) of the freeze-dried sewage sludge was weighed and wetted with 0.2 mol L⁻¹ of NH₄Cl solution (15 mL) and the mixture was thoroughly stirred using an Ultra Turrax in a centrifuge tube for 30 seconds. A mixture of n-hexane and acetone (50:50 v/v, 50 mL) was added to the wetted sludge and the sample was stirred for 5 minutes with the Ultra Turrax. The solvent on hydrated solids was separated by centrifuging the mixture at 4500 rpm and the supernatant was pipetted from the centrifuge tube.

A further portion of 0.2 mol L⁻¹ NH₄Cl solution (10 mL) and the n-hexane/acetone mixture (50:50 v/v, 25 mL) were added to the remaining solid; after the whole had been mixed for 5 minutes using the mixer, the tube was centrifuged again at 4500 rpm for 10 minutes. The solvent was pipetted from the tube. This extraction procedure was repeated a further time and the solid sludge was finally extracted with 50 mL of n-hexane/acetone mixture. All organic extracts were combined and washed successively with 250 mL and 100 mL portions of distilled water to remove the acetone. The remaining n-hexane layer was transferred to a rotary evaporator and concentrated to 10 mL.

In the concentrated extract, the fatty components were destroyed by saponification with 10 mL of potassium hydroxide solutions (400 g dissolved in 1 L water), together with 10 mL of ethanol, on a water bath at 90°C for 1 h. The resultant mixture was cooled and extracted in a separating funnel with n-pentane (20 mL). The extraction was repeated three times. The combined pentane extracts were washed with sodium sulphate solution (20 g L⁻¹) on a glass fibre filter, which was further washed with n-pentane (20 mL). Iso-octane (5 mL) was added to the sample and the extract was concentrated to 5 mL using a rotary evaporator. The sample was further diluted with n-pentane (30 mL) and transferred to the top of an alumina column filled with basic Al₂O₃ containing 6% (m/m) distilled water. The sample was eluted with n-pentane (60 mL). The eluate was concentrated on the rotary evaporator to 5 mL, quantitatively transferred to a graduated flask and diluted with iso-octane to 10 mL. A 1 mL aliquot of this solution was pipetted into a measuring cylinder together with 1 mL of the internal

standard 2,4 dichlorobenzyl tetradecylether (1.5 mg L^{-1}). The sample volume was reduced to 1 mL by evaporation under nitrogen at ambient temperature.

The final solution was injected ($2 \mu\text{L}$) onto a 50 m fused silica capillary GC column. The GC conditions are described in details elsewhere [21]. The calibrants (BCR CRM Nos 291, 293, 294, 295, 296, 297 and 298) and blanks were treated in exactly the same way as the samples. The recoveries of the CBs varied between 80% and 100%. The determination of each CB was verified to be completed within the linear range of the ECD detector.

The CVs for all seven CBs did not exceed 11% and there was no significant distinction between the 'within-bottle' CVs for each CB obtained from the replicate analysis of the contents of three separate bottles. The magnitude of the variances increased as the concentration decreased, with the exception of CB 180, mainly due to the method of clean-up used [21]. Thus, saponification of the organic extract for a prolonged period of time, at a too high temperature, has been shown to cause degradation of this CB [23]. Although the conditions of the analysis for these experiments were optimised as well as possible at the time, it is likely that this degradation contributed to the slightly higher variance. The between-bottle CVs for the analysis of the CBs taken from 15 separate bottles was less than 9%. There was no significant difference between the within-bottle and the between-bottle CVs [21,23].

The stability was tested at -18°C , $+20^\circ\text{C}$ and $+37^\circ\text{C}$ by analysing the material at the beginning of the storage period and at the end of the certification campaign (36 months after the first analysis). The same analytical method as the one used for the homogeneity study was applied. No instability could be demonstrated for any of the CB determined. It was observed that the repeatability of the methods was significantly better after three years, which was the result of the participation in interlaboratory studies [21,23].

10.4.3. Certification

The final determination of the CBs was performed by capillary gas chromatography with electron capture detection; mass spectrometry was used as complementary technique to confirm the identity of each of the CBs determined. Each participant had validated its method by performing experiments on recovery, extraction efficiency, procedure blanks and detector linearity. The seven individual CB calibrants were supplied to the participants as pure, crystalline CRMs from BCR (CRMs Nos. 291, 293, 294, 295, 296, 297 and 298). Each laboratory was requested to prepare separate calibration solutions of the appropriate concentration, in iso-octane, to calibrate the detector and lying within its linear range. The use of at least one internal standard was mandatory; the participants, however, were left free to select the internal standard(s) best suited to their methods. They had to verify that the selected compounds did not occur in the candidate reference material or did not interfere with compounds present in the material. A series of pure dichlorobenzylalkyl ethers (DCBEs) was made available to the participants but other internal standards were also accepted of which the list is given in the certification report [21] along with additional details on calibration procedures.

Details on the methods used (extraction, clean-up, separation) are given in the certification report [21]. Extraction was either carried out ultrasonically or by Soxhlet

using organic solvent, e.g. hexane, acetone, n-pentane, dichloromethane, following in some cases wetting of the material with ammonium chloride solution. Clean-up was performed e.g. by treatment with concentrated H_2S_4 followed by desulphurisation, column chromatography with e.g. activated alumina, separation on silica gel etc.; saponification was also used (with a solution of potassium hydroxide followed by pentane, hexane or iso-octane extraction), as well as Gel Permeation Chromatography (GPC). Capillary gas chromatography was used, identifying the CB compounds on the basis of their relative retention times. In all cases, at least two columns of different polarity have been used for quantification. For each CB, the participant selected the best suited column. Some participants used mass selective detection (MSD); in this case, the appropriate masses were monitored for the CBs and the internal standards. Quantification was either by peak area or by peak height.

The recoveries of the methods were determined by spiking the sewage sludge with known of each of the CBs. Spiking was either on a single level in triplicate or on three levels in at least singlefold. In case of recovery values over 100% no corrections have been on the final CB contents in the sewage sludge. In case of recovery values under 100% corrections have been made. Data for CBs with recoveries significantly differing from 100% were discussed and possibly rejected. When isotope dilution with labelled CBs was applied, results were not corrected for recovery (isotope ratio calculation principle). Recoveries ranged from ca. 70 to 110%.

During the certification, one participating laboratory identified a second chlorobiphenyl co-eluting with CB 138. This compound was CB 163, which could not be resolved from CB 138 on most common chromatographic phases, e.g. SE-30/SIL-5, SE-54/SIL-8 or SIL-19. It was also not possible to distinguish between the two compounds using electron impact mass spectrometry. The CB 163 could only be identified when using negative-ion chemical ionisation MS. The similar retention times of CB 138 and CB 163 were confirmed by Mullins et al. [24]. It was previously thought that this compound did not occur in significant concentrations in technical mixtures and/or environmental samples. However, these recent studies lead to an estimated contribution of CB 163 up to 25% in this case of the total ECD response of the two isomers. In view of this information, the mass fraction of CB 138 in the sludge is not given as a certified value. However, the data obtained were given as indicative information on the measurements made for the total CB 138 + CB 163 [21].

The identity of the other measured CBs was confirmed by GC-MS. The six other compounds (CBs 28, 52, 101, 118, 153 and 180) were certified and the values are given in Table 10.6 along with their uncertainties.

10.4.4. Participating laboratories

The material has been prepared by the Joint Research Centre, Environment Institute, of Ispra (Italy). The homogeneity and stability were verified by the Netherlands Institute for Fishery Investigations (RIVO) in IJmuiden (The Netherlands). The analyses for certification were performed by the following laboratories: CID-CSIC, Departamento de Química Analítica, Barcelona (Spain); Department of Agriculture and Fisheries for Scotland, Aberdeen (United Kingdom); Direção Geral da Qualidade do Ambiente,

TABLE 10.6

CERTIFIED CB CONTENTS IN SEWAGE SLUDGE, CRM 392

CB IUPAC No.	Certified value ($\mu\text{g kg}^{-1}$)	Uncertainty ($\mu\text{g kg}^{-1}$)	p
28	100	10	20
52	79	9	18
101	134	11	18
118	97	12	14
153	288	18	20
180	313	24	19

p: number of sets of results

Lisbon (Portugal); Instituto Químico de Sarria, Barcelona (Spain); Institut d'Hygiène et d'Epidémiologie, Brussels (Belgium); Institute for Marine Research, Bergen (Norway); IFREMER, Brest (France); Instituto Hidrográfico, Lisbon (Portugal); Istituto sull'Inquinamento Atmosferico, CNR, Roma (Italy); Joint Research Centre, Ispra (Italy); Laboratoire d'Hydrologie et Molysmologie Aquatique, Faculté de Pharmacie, Marseille (France); Laboratoire Municipal et Régional, Rouen (France); Milchwirtschaftliche Untersuchungs- und Versuchsanstalt, Kempten (Germany); National Food Agency, Søborg (Denmark); National Swedish Environment Protection Board, Solna (Sweden); Netherlands Institute for Fishery Investigations, RIVO, IJmuiden (The Netherlands); State Institute for Quality Control of Agricultural Products, RIKILT, Wageningen (The Netherlands); Vrije Universiteit Amsterdam, instituut voor Milieuvraagstukken, Amsterdam (The Netherlands)

10.5. PAHs IN SEWAGE SLUDGE

10.5.1. Introduction

Polycyclic aromatic hydrocarbons (PAH) possess a pronounced carcinogenic and mutagenic potential. Though they are also formed by natural process of coalification, the main source of their formation is due to human activities through the incomplete combustion of fossil fuel, e.g. coal, gasoline, diesel fuel and oil. Accordingly, PAHs are ubiquitous in the environment where at least some of them, due to their high persistence, may accumulate. National recommendations and regulations have set limits in some countries for the emission of benzo[a]pyrene as a typical representative of PAHs. Modern technical concepts (motor design, catalyst, optimised combustion) have recently resulted in a reduced PAH output per mass of combusted fuel.

Sewage sludge has been found to be a sink for PAH originating from both domestic households and industrial sources. Hence this matrix may reflect the actual environmental situation and can be used as a target matrix in a continuous monitoring of the PAH

pollution of the environment. To respond to the need of tool for quality control, a sewage sludge reference material certified for its PAH content was deemed necessary; the CRM 088 was certified for eight PAHs selected on the basis of their abundance and their biological activity (carcinogenicity and/or mutagenicity) [25].

10.5.2. Production of the candidate reference material

Centrifuged sewage sludge was obtained from a municipal waste water treatment plant of a large city (100 kg with dry matter content of 21% as mass fraction). The material was heat-sterilised (medium-pressure) at 134°C for three intervals of (1) 75 minutes, (2) 60 minutes and (3) 60 minutes by autoclavation. In between the material was allowed to cool for 60 minutes at ambient temperature. The material was dried in a climate chamber at 20°C under air while being crushed mechanically. This was performed in stainless steel-containers in which the material had been spread and frequently raked for 3 months. Finally the material was dried in a ventilated oven for 48 h at 40°C.

The material was then ground in a tungsten carbide hammer-mill and sieved. The fraction <90 µm (more than 90% of the total mass) was homogenised in a mixing drum; the fraction >90 µm being discarded. The material was filled into brown screw capped glass bottles under argon (10 g into each bottle). During the filling procedure, every 50th bottle was set aside for the homogeneity study.

Sub-samples of 1 g were taken for the analysis. The PAHs were extracted under reflux with toluene/acetone (1:1 v/v, 150 mL) for 30 minutes. The efficiency of the extraction procedure was tested by adding an internal standard (indeno[1,2,3-*cd*]fluoranthene) and was found to be 95–99%. An additional extraction with tetrahydrofurane did not enhance the PAH yield. The extract was evaporated to about 2 mL. A cyclohexane/dimethylformamide (DMF)/water (100 mL cyclohexane, 90 mL DMF and 10 mL water) partition was performed. The cyclohexane phase was discarded and the DMF/water phase diluted with 80 mL water. This phase was re-extracted with cyclohexane. The cyclohexane phase was evaporated to a volume of 2 mL and cleaned-up over a silica column (5 g, 9.1% water). The PAHs were eluted with 80 mL cyclohexane, which was evaporated afterwards to a volume of 0.1–0.5 mL. The obtained concentrate was placed on a Sephadex LH 20 column (10 g) and eluted with propanol-2. The fraction of 0–46 mL was discarded. The fraction from 46–170 mL was evaporated to a small volume and analysed by high resolution gas chromatography (on column injection) using a fused silica capillary column of which the conditions are described elsewhere [26].

No significant differences were found between the within- and between-bottle CVs. These CVs were below and in some cases slightly above the CVs of the GC-determination (replicate analysis of a cleaned extract of sewage sludge). The heteroatom in benzo[*b*]naphtho[2,1-*d*]thiophen affects the FID-response; this leads to the comparatively high CVs obtained for this compound. The data demonstrated that the small variation of the results was caused by the analytical method rather than by inhomogeneity of the material. Therefore, the sewage sludge was considered to be sufficiently homogeneous to serve as a CRM for PAHs.

The stability of the PAHs in the sewage sludge was tested over a period of 12 months

at -20°C , $+20^{\circ}\text{C}$ and $+40^{\circ}\text{C}$, using the same technique as for the homogeneity study. The long term reproducibility was assessed by analysing a raw extract prepared at the beginning of the storage period and stored at -20°C . The contents of PAHs did not show significant changes during the stability experiment at both -20°C and $+20^{\circ}\text{C}$. For benzo[*a*]anthracene, indeno[1,2,3-*cd*]pyrene and benzo[*a*]pyrene a decrease of the contents was observed at $+40^{\circ}\text{C}$ over the period of 12 months. During the preparation, the homogeneity and the stability studies, the material was always stored below $+15^{\circ}\text{C}$; on the basis of the stability study, and to avoid any instability risks, the material was stored at $+4^{\circ}\text{C}$. It is recommended that this CRM be stored at $+4^{\circ}\text{C}$ or below to avoid losses of PAHs.

10.5.3. Certification

The final determination of the PAHs was performed by capillary gas chromatography using flame ionisation or mass spectrometric detection, or by high-performance liquid chromatography using UV- and fluorescence detection. Each participant had validated its method by performing experiments on recovery, extraction efficiency, procedure blanks and detector linearity.

Participants were requested to use as much as possible the CRM calibrants from BCR [26]. The use of at least one internal standard was mandatory; the participants, however, were left free to select the internal standard(s) best suited to their methods. They had to verify that the selected compound(s) did not occur in the candidate reference material or did not interfere with compounds present in the material. Two internal standards were made available from BCR, namely indeno[1,2,3-*cd*]fluoranthene (CRM 267) and 5-methylchrysene (CRM 081R).

Details on the methods used (extraction, clean-up, separation) are given in the certification report [26]. Extraction was either carried out ultrasonically or by Soxhlet using organic solvent, e.g. cyclohexane, acetone, methanol, and toluene. Clean-up was performed with alumina cartridge or silica with an elution with e.g. cyclohexane, toluene, *n*-pentane etc. Also HPLC and TLC on silica were used for the clean-up. Capillary gas chromatography was used, identifying the PAH compounds on the basis of their relative retention times and, in case of mass selective detection, on the basis of their ion masses. In all cases, at least two columns with different stationary phases and different polarity have been used for quantification. For each PAH, the participant selected the best suited column.

Three laboratories determined the PAHs using HPLC with UV and fluorescence detection. All laboratories performing HPLC used the same column type, which has been developed exclusively for PAH determination. For UV-detection an efficient clean-up is a prerequisite as the detection method is not selective and thus will respond also to many co-eluting compounds. Due to the high fluorescence activity of the PAHs the fluorescence detection is less susceptible to such interferences. But also in this case care was taken to achieve a sufficient clean-up to remove all co-eluting quenching compounds. The PAHs were eluted with acetonitrile/water mixtures with a gradient that ranged from 50% acetonitrile to pure acetonitrile. The introduction into HPLC systems is much more reproducible than in GC systems and therefore only one laboratory

used an internal standard. Four laboratories used a mass spectrometric detector for quantitation. At least two ions per component were measured to verify the absence of other compounds. The laboratories used Quadrupole-MS detectors with low to medium resolution.

The recoveries of the methods were determined by spiking the sewage sludge with known of each of the PAHs at three different levels and comparing the expected and measured values. Recoveries ranged from ca. 60 to 107%.

The water mass fraction of the material varied considerably from 7.3 to 10.9% (one participant even reported values around 3%). The water content may change with the humidity of the ambient air; therefore, it must be measured at the beginning of each analytical session and corrections to dry mass must systematically applied.

There was evidence from two laboratories that a clean-up over magnesium silicate only was insufficient. This led to an unstable baseline and to results with a very high standard deviation (high chemical background).

The detectors to be used in HPLC (fluorescence or UV) were not sufficiently sensitive to determine benzo[*b*]naphtho[2,1-*d*]thiophene at concentrations occurring in this material. HPLC results could hence not be obtained.

No other difficulties were experienced and the eight PAHs were certified as listed in Table 10.7.

10.5.4. Participating laboratories

The material was prepared by the Joint Research Centre of Ispra (Italy) and the Biochemisches Institut für Umweltcarcinogene in Grosshansdorf (Germany) which also carried out the homogeneity and stability studies. The following laboratories participated in the certification: Biochemisches Institut für Umweltcarcinogene, Grosshansdorf (Germany); Institut Universitaire de Médecine et d'Hygiène du Travail, Lausanne (Switzerland); Istituto Nazionale per la Ricerca sul Cancro, Genova (Italy);

TABLE 10.7

CERTIFIED PAH CONTENTS IN CRM 088

Compound	Certified value ($\mu\text{g kg}^{-1}$)	Uncertainty ($\mu\text{g kg}^{-1}$)	p
Pyrene	2.16	0.09	12
Benz[<i>a</i>]anthracene	0.93	0.09	11
Benzo[<i>a</i>]pyrene	0.91	0.09	12
Benzo[<i>e</i>]pyrene	1.02	0.07	11
Benzo[<i>b</i>]fluoranthene	1.17	0.08	9
Benzo[<i>k</i>]fluoranthene	0.57	0.05	9
Indeno[1,2,3- <i>cd</i>]pyrene	0.81	0.06	12
Benzo[<i>b</i>]naphto[2,1- <i>d</i>]thiophene	0.42	0.05	7

p: number of sets of results

Rheinisch-Westfälischer, TÜV, Essen (Germany); Rijksinstituut voor de Volksgezondheid en Milieuhygiëne, Bilthoven (The Netherlands); SoPrA Protezione Ambientale, Milano (Italy); Technical Research Centre of Finland, VTT, Espoo (Finland); Università di Perugia, Perugia (Italy); Union Technique de l'Automobile, du Motorcycle et du Cycle, Montlhéry (France); Vlaamse Instelling voor Technologisch Onderzoek, Mol (Belgium); Vrije Universiteit Amsterdam, Instituut voor Milieuvraagstukken, Amsterdam (The Netherlands).

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Chapter 11

Other types of CRMs

11.1. COAL MATERIALS

11.1.1. Introduction

The considerable increase in the use of coal in the 1980's has caused considerable concern with respect to the protection of the environment by heavy metals (in dust and ashes) and consequently regulations concerning emission levels have been developed [1]. Despite the importance of measuring the polluting components in coal, analytical agreement between laboratories has been found to be poor in many instances [2,3]. The lack of accuracy and consequent poor comparability of data were the reason for not detecting the major source of pollution by mercury in certain areas, which was suspected to be through industrial emissions (e.g. electrolytic chlor-alkali production). Indeed, indirect mercury release via combustion of fossil fuels may contribute significantly to the total mercury load. A decision could not be made because the mercury contents used to calculate the indirect release differed so widely that the contribution of the combustion of fossil fuels to the total pollution could not be established accurately enough [4]. Reference materials were requested to BCR by the steel, coal and power industries as a necessary tool for quality control [5].

The major components of coal (C, H) are usually determined to assist the classification of coal and in establishing its economic value. Elements present in smaller, but still significant amounts, (N, S, Cl) are determined to establish the liability of the coal to produce corrosion in burners when used for power generation, or to assess the suitability of coal for carbonisation, where the sulphur content is of great importance and to take account of the effects of the combustion products of these elements in the environment.

The certifications described in the following sections were in some instances carried out as a follow-up of interlaboratory studies organised to improve the state-of-the-art of coal analysis; these exercises are described in detail elsewhere [3].

11.1.2. Trace elements in coal

11.1.2.1. Production of the material

The first coal to be certified (CRM 40) was prepared in 1982 and was certified for its contents of a range of elements (As, Cd, Co, Cr, F, Hg, Mn, Ni, Pb, Zn) [6]. The material consisted of a steam coal with an ash content of ca. 17%, which is often used in coal-fired power plants. About 100 kg of this coal were ground in a Retsch hammer mill. About 25 kg of the material with a particle size of 60–90 µm was sieved out and

homogenised for ca. 30 h in a mixing drum especially designed to avoid contamination of the coal. The material was stabilised by removing adherent water as much as possible. This was done by continuing the treatment in the mixing drum over three weeks and at regular intervals (e.g. once a day), replacing the argon in the mixing drum by dry argon. The temperature in the drum (up to 45°C) and these replacements resulted in a low water content and thus in a more stable material. The homogenised material was put into glass bottles provided with a soft polythene insert and a hard plastic screw cap. After 40 bottles had been filled, the homogenisation drum was closed again, the remaining material was mixed for about 10 minutes and then another 40 bottles were filled, and so on. Each bottle contained approximately 50 g of material. Care was taken to avoid contamination by air or dust during these operations.

Coal materials, especially those of high ash, are prone to segregation. The different phases present in the coal have different densities and different trace element contents. The homogeneity was verified by instrumental neutron activation analysis (INAA) [7]. Samples were irradiated with thermal neutrons in a tube container for 10 min, or for 40 h when mounted on a turntable. In the first case the neutron flux was kept constant. After an appropriate decay, the γ -energies of the samples were measured, using a Ge(Li) detector with 2 keV resolution and 10% relative efficiency. Peaks with $>5-10 \times 10^3$ counts were considered. For these peaks, the coefficient of variation to be expected from counting statistics and weighing could be estimated (CV_{est}). A comparison between CV_{est} and the CV actually obtained (CV_{obt}) gave an indication of the maximum inhomogeneity, as other factors which contribute to CV_{obt} , such as irregular loading of the counting vials and variations in thickness, packing or density of the samples in the vials are not considered. The results showed that the maximum inhomogeneity at the 100 mg level, depending on the element, gave rise to coefficients of variation of 0–5%. In this type of trace analysis, normal coefficients of variations are 10–15%; it was hence concluded that the between-bottle homogeneity was sufficient for such a sample [6].

The stability of the material was monitored over the period 1978–1984, using various analytical techniques, e.g. INAA for As, Co, Cr, Mn and Zn, ETAAS for Cd, Hg and Pb, and IDMS for Cd, Ni and Pb. No change in the contents of these trace elements with time could be detected over the stability study [6].

11.1.2.2. Certification

The techniques of final determination are summarised in Table 11.1. Sample pretreatment methods were e.g. dry ashing or pressurised digestion with combination of acids. Techniques such as ICP-AES or ETAAS with Zeeman background correction were not yet popular at the time of the certification campaign [8].

A good agreement was generally obtained for all elements certified. The certified values, along with their uncertainties are given in Table 11.2.

11.1.2.3. Participating laboratories

The material was prepared by the Joint Research Centre of Ispra (Italy). The following laboratories participated in the certification: Aluminium Pechiney, Voreppe (France);

TABLE 11.1

SUMMARY OF TECHNIQUES USED IN THE CERTIFICATION OF THE COAL CRM 40

Element	Techniques
As	ETAAS, HGAAS, INAA, PAA, SPEC, TITR
Cd	ETAAS, FAAS, INAA, PAA, RNAA
Cr	ETAAS, FAAS, INAA, PAA, RNAA
Co	FAAS, ETAAS, INAA, PAA
F	POT, SPEC
Mn	ETAAS, FAAS, ICP-AES, INAA, PAA, SPEC
Hg	CVAAS, CVAFS, PAA, RNAA
Ni	ETAAS, FAAS, ICP-AES, PAA, RNAA
Pb	ETAAS, FAAS, ICP-AES, IDMS, PAA
Zn	ETAAS, FAAS, INAA, PAA, RNAA

TABLE 11.2

CERTIFIED VALUES IN CRM 40

Element	Certified value (mg kg ⁻¹)	Uncertainty (mg kg ⁻¹)	p
As	13.2	1.1	12
Cd	0.11	0.02	14
Co	7.8	0.6	13
Cr	31.3	2.0	14
F	111.4	8.5	11
Hg	0.35	0.06	15
Mn	139	5	14
Ni	25.4	1.6	18
Pb	24.2	1.7	18
Zn	30.2	1.9	17

p: number of sets of results

British Carbonization Research Association, Chesterfield (United Kingdom); Bundesanstalt für Materialprüfung, Berlin (Germany); Centre d'Etudes Nucléaires de Saclay, Gif-sur-Yvette (France); Centro di Radiochimica e Analisi per Attivazione, CNR, Pavia (Italy); Centro Sperimentale Metallurgico spA, Roma (Italy); Corex Laboratories, Rotherham (United Kingdom); Energieonderzoek Centrum Nederland, ECN, Petten (The Netherlands); Hoesch Hüttenwerke, Dortmund (Germany); Hoogovens, Ijmuiden (The Netherlands); IRSID, Maizières-lès-Metz (France); Joint Research Centre, Ispra (Italy); Kernforschungsanlage, Institut für Chemie, Jülich (Germany); Laborlux, Esch-sur-Alzette (Luxemburg); Ruhrkohle AG, Essen (Germany); Universitaire Instelling Antwerpen, Departement Scheikunde, Wilrijk (Belgium);

Universiteit Gent, Instituut voor Nucleaire Wetenschappen, Gent (Belgium); Yorkshire Regional Laboratory, NCB, Rotherham (United Kingdom).

11.1.3. Minor and Major elements in three coals

11.1.3.1. Introduction

Three coals were selected as reference materials, representative of materials used in the three major coal consuming or coal processing industries, namely gasification, carbonisation and power generation [9]. The gas coal (CRM 180) was a high-volatile, weakly coking coal of moderately high ash content; its properties were typical of coals formerly used in the coal-gas industry. The coking coal (CRM 181) was a high-volatile, strongly coking coal of low ash and moderate sulphur content; its principal use is an additive in blends of coal used for carbonisation where its high swelling properties and high fluidity are useful in improving the quality of coke. The steam coal (CRM 182) was a medium-volatile coal of high ash content, similar to many coals used for power generation.

11.1.3.2. Production of the reference materials

Gross samples of lump coal were selected by manual picking from the steam coal. Initial size reduction to a particle size of approximately 12–15 mm was carried out by manual crushing using a manganese steel pestle and mortar. Further size reduction was carried out in two stages using crushing rolls (chrome steel) to yield a product of particle size of approximately 1 mm. Final size reduction to about 0.2 mm particle size was carried out using either a cast-iron disc mill or a tungsten carbide centrifugal mill. This material was sized using standard wire sample of particle size between 63 and 212 μm . Particles of a size greater than 212 μm were re-crushed and returned to the bulk sample; material below 63 μm in size was discarded. The sieving operation was carried out concurrently with the size reduction, all processes being carried out within the overall temperature interval of 17–20°C.

The yield of between 21 and 23 kg of the final product was stored under argon prior to sample division in a rotary sample divider. This operation yielded 20 sub-samples each of approximately 1 kg. Each sub-sample was protected from oxidation by an argon atmosphere. The samples for analysis were transferred to glass ampoules, previously dried in nitrogen at 28°C in a special box over dry (blue) silica gel. The method of filling the ampoules varied with each coal as follows:

- Coal 180: by manual filling after re-mixing the sample in a mixing drum filled with dry argon, which was replaced every 3 h;
- Coal 181: incremental manual sampling (50 increments per ampoule) from the coal spread out in a thin layer on glazed paper;
- Coal 182: sample division by rotary sample divider and direct transfer to the ampoule.

The filled ampoules were evacuated, argon (cleaned over gold) being then admitted to restore the pressure to atmosphere. This procedure was repeated. After cleaning the neck of the ampoule with a cotton wool swab, it was sealed with a small gas torch. The

distance between the upper level of the coal sample and the point of sealing was at least 12 cm. As the operation lasted for only 12–20 seconds, heat transfer to the coal surface did not take place and no oxidation of the coal occurred. Ampoules were filled in batches of about forty.

Although the particle size range of an analytical sample of coal is relatively small, segregation can occur in the ampoules upon standing and following transportation. Before analysis, the whole sample should be re-homogenised e.g. by crushing to a particle size of less than 180 μm and thoroughly mixing before weighing, or by further grinding or other suitable techniques. For trace elements, the homogeneity was verified by instrumental neutron activation analysis (INAA). The samples of different masses were irradiated with thermal neutrons using two methods: (1) in a tube container for about 10 h and (2) placed on turntable for about 40 h. In method (1) the stability of the flux was verified during the whole irradiation period (iron to monitor the flux); the samples on the turntable in method (2) received the same integrated flux of the same neutron energy spectrum. The irradiation time was the same for all the samples examined by method (1). The starting and finishing points and the decay time (12 h) were measured to enable the necessary corrections for the decay of the resulting isotopes to be made. The activity was measured for both methods (1) and (2) with a γ -spectrometer using a Ge(Li)-detector with 2 keV resolution (at 660 keV) and about 10% relative efficiency. The detector was coupled to an automatic sampler and a multi-channel analyser. The decay time for the samples examined by method (2) was 14 days.

In the evaluation only peaks of elements with minimal 5000–10000 counts were considered. The overall uncertainty in the results of these measurements is caused by various factors of which some, like random weighing errors and counting errors, are calculated. For these peaks, the coefficient of variation to be expected from counting statistics and weighing could be estimated (CV_{est}). A comparison between CV_{est} and the CV actually obtained (CV_{obt}) gave an indication of the maximum inhomogeneity, as other factors which contribute to CV_{obt} , such as irregular loading of the counting vials and variations in thickness, packing or density of the samples in the vials are not considered. No inhomogeneity was detected at the level of intake of 100 mg, i.e. there was no detectable between-ampoule inhomogeneity. Not all trace elements of interest can be studied in this way. However, many other elements are usually dispersed in a way similar to the tested elements (e.g. As-P-Sb, Zn-Hg-Cd etc. [10]). This procedure of homogeneity testing has been described in more detail elsewhere [7,11].

A bottled coal has shown to be stable with respect to trace element contents for over six years [8]. The stability of major components, ash contents and trace components in a coal confined in a sealed ampoule under argon placed in a special container should, in principle, be adequate for reference materials; stability checks were, however, performed over two years and confirmed the long term stability for the three CRMs [9].

11.1.3.3. Certification

The techniques of final determination are summarised in Table 11.3. Sample pretreatment methods were e.g. dry ashing or pressurised digestion with combination of acids.

A generally good agreement was obtained among the laboratories, with the exception

TABLE 11.3

SUMMARY OF TECHNIQUES USED IN THE CERTIFICATION OF THE COAL CRMS 180, 181 AND 182

Element	Techniques
C and H	CRSTC, GRAV, PCSTC
Cl	BCIC, HTAT, EDTA, EDVT, INAA, SPEC, TCPT, WCPT
N	CRSTC, KJEL, PCSTC
As	EDXRF, ETAAS, HGAAS, HICP, INAA, RNAA, SPEC, XRF, ZETAAS
Cd	DPASV, ETAAS, IDMS, ICP-AES, ZETAAS
Mn	ETAAS, FAAS, ICP-AES, INAA, XRF
Hg	CVAAS, HICP, RNAA
Pb	DPASV, EDXRF, ETAAS, FAAS, IDMS, PAA, ZETAAS
Se	EDXRF, ETAAS, HGAAS, HICP, INAA, RNAA, XRF, ZETAAS
V	ETAAS, FAAS, ICP-AES, INAA, ZETAAS
Zn	DPASV, EDRXF, ETAAS, FAAS, ICP-AES, IDMS, INAA, XRF

of some outliers which were due to contamination problems or calibration errors. A poor agreement, however, hampered the certification of the manganese content in the CRM 181, and of the hydrogen, arsenic and lead contents in the CRM 182.

One laboratory used ammonium chloride as a calibrant for chlorine determination which was unsatisfactory owing to an instability in irradiation. In general it is necessary to irradiate the sample in sealed vials to avoid a possible volatilisation of chlorine or hydrochloric acid, which can be formed in the irradiation stage.

Another set of results was rejected for arsenic because of possible losses in the dry ashing; ashing aids (e.g. MgO or Eschka-mixture) were indeed not added.

Combustion difficulties were experienced by one laboratory for selenium in CRM 181. The participant added quartz to decrease the high temperature of the combustion and it could not be excluded that a part of the selenium had reacted with the additive at high temperature or that a part of the selenium had been adsorbed on the quartz.

A more detailed discussion of the results is given in the certification report [9]. The certified values are given in Table 11.4, along with their uncertainties.

11.1.3.4. Participating laboratories

The material was prepared by the British Carbonization Research Association in Chesterfield (United Kingdom). The homogeneity studies were carried out by the CNR, Centro de Radiochimica e Analisi per Attivazione in Pavia (Italy), the Dutch Centre for Coal Specimens (SBN) in Eindhoven (The Netherlands), the Gesellschaft für Strahlen- und Umweltforschung in Neuherberg (Germany), and the TNO Division of Technology for Society in Zeist (The Netherlands). The following laboratories participated in the certification: Atochem, Levallois (France); Central Electricity Generating Board, Nottingham (United Kingdom); Centre d'Etudes et Recherches des Charbonnages de France, Verneuil-en-Halatte (France); CNRS, Service Central d'Analyse, Vernaison

TABLE 11.4

CERTIFIED VALUES IN CRMS 180, 181 AND 182 (VALUES AND UNCERTAINTIES GIVEN IN g kg^{-1} FOR C, H, N AND Cl AND IN mg kg^{-1} FOR THE OTHER ELEMENTS)

Element	CRM 180	CRM 181	CRM 182
C	760.1 ± 2.1	848.9 ± 1.7	732.9 ± 2.2
H	50.4 ± 0.6	54.0 ± 0.6	(42.2 ± 1.5)
N	14.4 ± 0.3	17.8 ± 0.4	16.36 ± 0.30
Cl	0.593 ± 0.026	1.38 ± 0.05	3.70 ± 0.07
As	4.23 ± 0.19	27.7 ± 1.2	(1.47 ± 0.28)
Cd	0.212 ± 0.011	0.051 ± 0.003	0.057 ± 0.004
Mn	34.3 ± 1.1	(2.82 ± 0.29)	195 ± 6
Hg	0.123 ± 0.008	0.138 ± 0.011	0.040 ± 0.007
Pb	17.5 ± 0.5	2.58 ± 0.16	(15.3 ± 1.2)
Se	1.32 ± 0.06	1.15 ± 0.05	0.68 ± 0.07
V	19.3 ± 0.6	12.0 ± 0.4	24.3 ± 1.0
Zn	27.4 ± 1.1	8.4 ± 0.6	33.3 ± 1.5

The values in brackets are given as indicative values (not certified).

(France); Centro di Radiochimica e Analisi per Attivazione, CNR, Pavia (Italy); CISE SpA, Segrate (Italy); Ecole Nationale Supérieure, Laboratoire de Chimie Minérale, Strasbourg (France); Eniricerche, S. Donato Milanese (Italy); Ente Nazionale per l'Energia Elettrica (ENEA), Direzione Studi e Ricerche, Pisa (Italy); Energieonderzoek Centrum Nederland, ECN, Petten (The Netherlands); Hoogovens, IJmuiden (The Netherlands); Institut National des Industries Extractives, Liège (Belgium); Isotopcentralen, Copenhagen (Denmark); Istituto Superiore di Sanità, Roma (Italy); Keuring van Electrotechnische Materialen N.V. (KEMA), Arnhem (The Netherlands); Kon. Shell Laboratorium, Amsterdam (The Netherlands); Ministère des Affaires Economiques, Laboratoire Central, Brussels (Belgium); National Coal Board, Mansfield (United Kingdom); TNO Division of Technology for Society, Zeist (The Netherlands); Nordjyllands Elektricitetsforsyning Vendsysselvaerket (NEFO), Vodskov (Denmark); Risø National Laboratory, Isotope Division, Roskilde (Denmark); Ruhrkohle AG, Essen (Germany); Stazione Sperimentale per I Combustibili, S. Donato Milanese (Italy); Studiecentrum voor Kernenergie, SCK/CEN, Mol (Belgium); Universiteit Ghent, Instituut voor Nucleaire Wetenschappen, Gent (Belgium); Universität Ulm, Lab. Analytische Chemie, Ulm (Germany).

11.1.4. Sulphur in six coals

11.1.4.1. Introduction

The sulphur content of coal is of considerable significance in relation to its industrial use. In combustion processes, such as power generation, sulphur compounds in the

waste gases are responsible for corrosion phenomena and, more importantly, are associated with the considerable environmental problems arising from the deposition of acidic materials from the atmosphere. In metallurgical processes, coal sulphur, or more frequently the sulphur retained in the coke after carbonization, has an important bearing both on the quality of the resultant metal and on the economics of the process. There is a wide and disparate literature, stretching back some eighty years relating to the nature of the occurrence of sulphur in coal and methods of its removal. In general terms, sulphur occurs in coal in two principal forms; mineral, mainly as pyrite or marcasite, and complex organic, linked to the carbon structure of the coal.

Because of the adverse effects of sulphur, a vast amount of effort has been directed towards reducing the sulphur content of coal. Mineral sulphur can be partially removed by cleaning processes, which are in widespread use, but organic sulphur, though it can be partially or entirely removed in laboratory or pilot scale processes, presents an intractable problem of removal on a large scale. For example, in coals otherwise suitable for carbonisation, processes which remove organic sulphur leave a coal residue, the coking properties of which have been destroyed.

For these reasons, the determination of sulphur in coal is of considerable importance and a representative selection of a wide range of industrial coals has been made with the aim to establish them as coal reference materials (CRMs 331, 332, 333, 334, 335 and 336) [12].

11.1.4.2. Production of the candidate reference materials

A suitable amount of lump coal of each material was progressively crushed and divided at ambient temperature under argon to provide a final batch of 20 kg of material passing a sieve with apertures of 0.125 mm. Then, after manual homogenisation in a sequence of steps the sample size was riffled down to 20 g (1000 samples). Each of the 20 g portions was finally loaded into vials, using the spinning riffling technique. The vials were evacuated and refilled with Ar. Heating of the coal during this process was avoided.

The homogeneity (within- and between-bottles) was verified using the method ISO 351 — Coal and Coke, Determination of Sulphur / High-temperature Combustion Method. The coefficient of variation of the within and the between homogeneity tests did not show any significant difference and were always below 1% which is less than 1/3 of the uncertainty obtained in the certification exercise. All materials were hence considered to be of suitable homogeneity for use as reference materials.

The stability of sulphur was verified at ambient temperature over the certification period (8 months). The results showed that the stability of the samples kept in sealed ampoules under argon was good.

11.1.4.3. Certification

The following techniques of determination were used in the certification:

- Heating with Eschka-mixture ($\text{MgO}/\text{Na}_2\text{CO}_3$) at 800°C and extraction of residue with dilute acid, followed by gravimetry;

- Ignition for 2 h at 800°C (Eschka method) and extraction of residue with water, followed by ICP-AES detection;
- Addition of ^{34}S spike, digestion with $\text{MgNO}_3/\text{HNO}_3$, dissolution in HCl , precipitation with Ba solution, fusion with carbonate, reduction with $\text{HI}/\text{H}_3\text{PO}_3$, distillation, conversion to As_2S_3 , detection by MS of relative masses 107 and 109;
- combustion in oxygen at 1250°C using FePO_4 as catalyst, followed by acidimetric titration, ion chromatography or titrimetry with $\text{Na}_2\text{B}_4\text{O}_7$;
- pressurised digestion with HNO_3/HF at 190°C for 9 h followed by ICP-AES detection;
- tube combustion in oxygen at 1450°C followed by titrimetry with $\text{Na}_2\text{B}_4\text{O}_7$;
- combustion in O_2/H_2 mixture (Wickbold combustion) followed by titrimetry with Ba^{2+} using thorin as indicator.

A large proportion of the results was obtained using the ISO 334 method (Sulphur in coal, determination by the Eschka method) using either acidic or aqueous extraction after the ignition of the coal with an Eschka mixture, or the ISO 351 method (as used in the homogeneity study). These methods are virtually identical with corresponding British, French or German national standards for the determination of sulphur in coal. Considerable technical expertise is required for the proper execution of the Eschka determination, particularly in the ignition conditions where strict control must be exercised over the furnace temperature, degree of ventilation of the furnace and the duration of the ignition stage. In addition, correct conditions of acidity must prevail during the removal of iron from the solution and the conditions under which the sulphur is precipitated as barium sulphate must be optimised. As described above, a number of alternative methods were also used.

For CRMs 331 and 333, the IDMS method used encountered a high standard deviation which was higher than normal. This was attributed to the fact that the laboratory only used manual shaking to re-homogenise the content of the ampoule (instead of e.g. crushing or grinding as it is recommended for this type of sample). The results obtained were, otherwise, generally in agreement. The certified values of sulphur in the six CRMs are given in Table 11.5.

TABLE 11.5

CERTIFIED VALUES OF SULPHUR IN CRMS 331, 332, 333, 334, 335 AND 336

CRM	Certified value (g kg ⁻¹)	Uncertainty (g kg ⁻¹)	p
CRM 331	4.99	0.10	11
CRM 332	9.61	0.17	12
CRM 333	13.44	0.26	12
CRM 334	16.09	0.19	12
CRM 335	50.8	0.6	14
CRM 336	32.90	0.26	14

p: number of sets of results

11.1.4.4. Participating laboratories

The coals were obtained from the Dutch Centre for Coal Specimens (SBN) in Eygeshoven (The Netherlands). The stability was verified by EOLAS, the Irish Science and Technology Agency in Dublin (Ireland). The following laboratories participated in the certification: British Coal, Burton-on-Trent (United Kingdom); British Gas plc, West-Midlands (United Kingdom); Centre d'Etudes et de Recherches des Charbonnages de France, Verneuil-en-Halatte (France); CISE SpA, Segrate (Italy); COMEXAL, Antwerp (Belgium); Empresa Nacional de Electricidad, Madrid (Spain); Energieonderzoek Centrum Nederland, Petten (The Netherlands); EOLAS, The Irish Science and Technology Agency in Dublin (Ireland); Ets. Gordinne & Cie, Rozenburg (The Netherlands); Hoogovens Groep b.v., IJmuiden (The Netherlands); Keuring van Electrotechnische Materialen N.V. (KEMA), Arnhem (The Netherlands); Ministère des Affaires Economiques, Laboratoire Central, Brussels (Belgium); Ruhrkohle AG, Essen (Germany).

11.1.5. Fluorine in coal

11.1.5.1. Introduction

Coal and fly ash are currently monitored to control air and ground water pollution, to evaluate mass balance in coal production and as indirect control of emission (based on the differences in contents between coal and fly ash). Some areas suffer from a severe pollution by emission of fluorine to the atmosphere and its consequent transport into the ground water. Chlorine is responsible for burner corrosion and has to be determined to assess the risk; monitoring of these elements under good quality control implies that CRMs of coal and fly ash should be available. A new coal reference material (CRM 460) has hence been prepared with the objective to certify both F and Cl [13,14]; as described below, only fluorine could be certified, owing to an in-homogeneity detected for Cl.

11.1.5.2. Production of the reference material

About 50 kg of South African Rietspruit coal, originating from the SBN (Steenkool Bank Nederland) collection, was first ground under constant cooling with liquid nitrogen to a particle size of less than 0.2 mm. Then, in a sequence of steps, the main sample was divided into sub-samples using the spinning riffling technique. The coal sub-samples were bottled under a dry air atmosphere: 1200 bottles were filled each with 40 g of coal. The bottles were evacuated and refilled with nitrogen. Heating of the coal during this process was avoided.

The homogeneity has been verified at the level of intake of 0.25 g. The method uncertainty was measured by seven replicate analysis of dissolutions of a coal CRM (NBS 1632a). F and Cl were determined by ion chromatography after oxygen combustion and absorption in water cooled in ice. No in-homogeneity was detected for fluorine. For Cl, a significant difference between the CV of the method and the CV between-bottles was demonstrated; the very low Cl content led to a large uncertainty of measurements which could, however, not explain the differences observed in the within- and

between-bottles CVs; consequently, the material was not considered to be suitable for the certification of this element [14]. The stability was verified over a period of 12 months at -20°C , $+20^{\circ}\text{C}$ and $+40^{\circ}\text{C}$. No instability was detected for fluorine in the coal reference material [14].

11.1.5.3. Certification

The techniques used were mainly based on oxygen combustion, pyrohydrolysis or fusion with Na_2CO_3 followed by ion selective electrode or ion chromatography. Neutron activation analysis with fast neutrons was also used by one laboratory. A detailed description of the methods is given in the certification report [13].

At the technical meeting, oxygen bomb combustion was considered doubtful for the determination of F in coal; this method was tested by KEMA using a wide variety of coal samples and the results obtained showed a wide range of F recoveries. The same was observed in an ISO-framework where laboratories worldwide confirmed this finding [14]. This method is recognised to be suitable for some coals but problems are likely to occur in some cases. This method being in doubt, results were only accepted for certification if the total destruction for F was confirmed by analysis of the combustion residue. The lack of control of the method was confirmed by a high spread of results obtained with this procedure. Therefore, it was agreed that this method, although it can be applied successfully in some cases, could not be used for the purpose of certification without confirmation of the residue.

One participant verified the residue (after combustion of the coal as previously carried out and fusion of the remaining slag with Na_2CO_3) and found that fluorine was retained to a value corresponding to $(15.0 \pm 2.3) \text{ mg kg}^{-1}$ of F which was taken into account in the recalculated set of data presented.

Another participant verified also the residue by dissolving the slag with H_2SO_4 but the amount of F found could not justify the low results previously attributed to an incomplete dissolution (ca. 3.4 mg kg^{-1} of F was found in the residue). However, this laboratory repeated the F determination by changing the buffer (sodium citrate bihydrate and potassium nitrate instead of acetic acid/sodium chloride/CDTA) and increasing its concentration to mask all complexing elements (e.g. Al). A higher value was found $((198.4 \pm 3.7) \text{ mg kg}^{-1})$ which was suggested to be used as an indicative value by the participant.

The certified value of fluorine and its uncertainty are $(225 \pm 6) \text{ mg kg}^{-1}$, as obtained by 12 laboratories (8 using ISE, 3 using IC and 1 using FNAA). As mentioned above, chlorine could not be certified owing to suspicion of an inhomogeneity; an indicative value of $(59 \pm 8) \text{ mg kg}^{-1}$ was obtained (corresponding to the mean and standard deviation of 15 sets of results).

11.1.5.4. Participating laboratories

The coal CRM was provided by the European Centre for Coal Specimens, SBN, in Egelshoven (The Netherlands). The homogeneity and stability studies were performed by KEMA N.V. in Arnhem (The Netherlands). The following laboratories participated

in the certification campaign: Bundesanstalt für Materialsforschung- und Prüfung, Berlin (Germany); CNRS, Service Central d'Analyse, Vernaison (France); Danish Technological Institute, Hasselager (Denmark); Energieonderzoek Centrum Nederland, Petten (The Netherlands); ELSAM, Aabentaa (Denmark); ENEL, Pisa (Italy); Gordinne & Cie, Rozenburg (The Netherlands); INERIS, Verneuil-en-Halatte (France); I/S Midtkraft, Skødstrup (Denmark); KEMA N.V., Arnhem (The Netherlands); Laborelec, Linkebeek (Belgium); Ministère des Affaires Economiques, Laboratoire Central, Brussels (Belgium); Ruhrkohle AG, Essen (Germany); Stazione Sperimentale per i Combustibili, San Donato (Italy); Technical Research Centre, VTT, Espoo (Finland); TÜV, Essen (Germany); Universiteit Gent, I.N.W., Gent (Belgium); Universidad de Oviedo, Departamento de Química Física y Analítica, Oviedo (Spain); University of Plymouth, Department of Environmental Sciences, Plymouth (United Kingdom).

11.2. ASH MATERIALS

11.2.1. Introduction

As mentioned in previous sections, trace element contents are not determined only in coal but also in slags, ashes etc. Fly ash being a combustion product which is formed in large amounts becomes increasingly important since pulverised coal combustion yields about 80% of all the ash in the form of fly ash. Generally, fly ash consists of particles with a diameter of 1–100 μm . These particles are trapped from the combustion gases by electrostatic precipitations (efficiency more than 98%) or by cloth filters (efficiency more than 99%). These efficiencies are high but the risk of air pollution still exists especially in the vicinity of a power station. For example, a 600 MW power station will exhaust 1000 tons a year into the air. The particles passing the filters most of the time are the smaller ones of which the contents of heavy metals are above average.

The major part of the fly ash is retained on the filters. Dumping of these large amounts is hardly possible, especially when there is a risk of contamination of ground water and the like. Possible uses of the material are in building and constructing (cement, concrete etc.). In order to decide upon an application of the material, the contents of toxic substances should be known which require a high degree of accuracy.

11.2.2. Trace elements, fluorine and chlorine in ash

11.2.2.1. Introduction

The certification of a range of trace elements has been certified in a reference material of fly ash obtained from the combustion of pulverised coal (CRM 038) in 1982 [15,16]. Following requests made by the coal industry and control laboratories, this material has been additionally certified for its content of F and Cl in 1994, following two intercomparisons which involved 14 laboratories (dealing with fluorine solutions with increasing concentrations) [17].

11.2.2.2. Production of the reference material

A batch of 150 kg of fly ash was collected at the electrostatic precipitator of a pulverised coal-fired power station in Belgium where the flue gases are directly fed to the precipitator without previous setting or filtering. Using coal with a typical ash content of 25% w/w, the hourly fly ash production is up to 7 tons. The collected quantity of 150 kg represented an output of approximately two minutes. The temperature of the ash at the collection port was about 80°C. The short duration of collection and storage was in favour of the homogeneity of the material.

Most of the particles collected had a size in the range of 0.5 to 40 µm. Contamination by supporting materials such as filters, fibres, insects etc. is highly unlikely due to the way of collecting the material. The collected material was packed into three dry propylene containers of 40 L, which were previously thoroughly cleaned with distilled water, alcohol and ether. The particles with a size larger than 10 µm were removed using air classification techniques. For this purpose a first coarse cut was made on a Hosokawa classifier. Afterwards, the fine product was further treated on an Alpine classifier. To ensure homogeneity the sample was then mixed in a Nauta mixer.

A subdivision of the remaining batch of about 25 kg was achieved in two stages. A chute riffler was used to split the batch in eight portions. Each of these portions was then passed through a spinning riffle which further subdivided the material in 2500 samples of ca. 10 g. Each sample was put into a brown glass bottle with a plastic screw stopper.

Fly ash of this type with small particles may contain a good part of the mercury present in the element form which is adsorbed to the particle surface [16]. Mercury vapour can migrate through the plastic cap and stopper. So for the purpose of stability of the finally certified mercury content, it was decided to put the material into hard glass (Duran 50) sealed ampoules, prior to the certification analyses. For this purpose, the content of the brown bottles was re-homogenised during seven days in a special mixing drum. Drying was carried out by replacing the gas in the drum every 6 h by dried argon. The samples to be put into the ampoules were taken out of a plexiglass mixing drum and brought directly into the pre-cleaned ampoules. After having taken about 40 aliquots, mixing was continued during at least 15 minutes. Approximately 6 g was put into the ampoules before sealing. The wall of the ampoule near the sealing point was cleaned from possibly adhering material. The ampoule was sealed at a distance of at least 12 cm above the surface of the fly ash in the ampoule using a small hot flame. The sealing took less than 10 seconds. Previous measurements have shown that the temperature in the ampoule at the fly ash surface and below did not change for more than 2°C. During the entire ampouling operation, greatest care was taken to avoid contamination.

The homogeneity has been checked at three occasions: once prior to the inter-comparison work and the first part of the certification, once after the ampouling prior to the second part of the certification, and once at the stage of the F and Cl certification. The first series of tests were carried out by instrumental neutron analysis (INAA). Samples were irradiated with thermal neutrons in a tube container for 10 min, or for 40 h when mounted on a turntable. In the first case the neutron flux was kept constant.

After an appropriate decay, the γ -energies of the samples were measured, using a Ge(Li) detector with 2 keV resolution and 10% relative efficiency. Peaks with $>5-10 \times 10^3$ counts were considered. For these peaks, the coefficient of variation to be expected from counting statistics and weighing could be estimated (CV_{est}). A comparison between CV_{est} and the CV actually obtained (CV_{obt}) gave an indication of the maximum in-homogeneity, as other factors which contribute to CV_{obt} , such as irregular loading of the counting vials and variations in thickness, packing or density of the samples in the vials are not considered. The results showed that the maximum in-homogeneity at the 50 mg level, depending on the element, gave rise to coefficients of variation of less than 5%. In this type of trace analysis, normal coefficients of variations are 10–15%; it was hence concluded that the between-bottle homogeneity was sufficient for such a sample for certification of trace element contents [15]. The homogeneity was further verified for F and Cl which were determined by ion chromatography after oxygen combustion and absorption in water cooled in ice. Although the method was not accepted for certification (see section on evaluation of results), its repeatability was considered to be sufficient to assess the homogeneity of the material. For this fly ash containing a high percentage of hollow spheres, there was an increase in the analytical difficulties, particularly in the destruction step with pyrohydrolysis which led to assume that differences in the within- and between-bottle CVs were rather due to analytical uncertainties of the method used than to an in-homogeneity of the material; this was supported by the results of the certification in which a CV of 2.8% was obtained between laboratories. Consequently, the material was considered to be homogeneous at least at a level of 0.25 g and above.

The material has been analysed for several parameters, including the trace elements to be certified, over a period of about four years and no appreciable variations were found [15]. Further checks carried out after 12 years indicated that the reference material was still stable which is certainly thanks to its storage in ampoules [17]. The stability of F and Cl was tested over a period of 12 months at -20°C , $+20^\circ\text{C}$ and $+40^\circ\text{C}$. No instability could be demonstrated for these two elements.

11.2.2.3. Certification

The methods used various types of sample pretreatments, e.g. digestion with HNO_3 , HCl and HF either at low temperatures or in a pressurised bomb, HClO_4 and H_2SO_4 followed by HF, pressurised bomb destruction including HClO_4 or H_2SO_4 followed by evaporation to dryness of HNO_3 and fusion with Na_2O_2 or Na_2CO_3 followed by repeated additions of HCl or HNO_3 and evaporation to dryness. The techniques of final determination used for the certified trace elements are listed in Table 11.6.

For F and Cl, the techniques used were mainly based on oxygen combustion, pyrohydrolysis or fusion with Na_2CO_3 followed by ion selective electrode or ion chromatography. Neutron activation analysis with fast neutrons was also used by one laboratory for F, as well as visible light spectrometry; the latter technique was also used for Cl as well as argentometric titration. A detailed description of the methods is given in the certification report [13].

A generally good agreement was obtained for the different trace elements certified

TABLE 11.6

SUMMARY OF TECHNIQUES USED IN THE CERTIFICATION OF THE FLY ASH CRM 038

Element	Techniques
As	HGAAS, ICP-AES, INAA, PAA, SPEC
Cd	DPASV, ETAAS, IDMS, RNAA
Co	FAAS, ICP-AES, INAA, PAA
Cu	DPASV, FAAS, ICP-AES, INAA
Fe	ASV, FAAS, ICP-AES, INAA, PAA, SPEC, TITR
Mn	FAAS, ICP-AES, INAA, PAA, SPEC
Hg	CVAAS, ETAAS, RNAA
Na	FAAS, FAES, ICP-AES, INAA, PAA
Pb	DPASV, ETAAS, FAAS, ICP-AES, IDMS, PAA, XRF
Zn	ASV, FAAS, ICP-AES, INAA, PAA

(with the exception of some sets of results which were rejected for technical reasons). For the determination of water-soluble sulphate, several widely varying techniques of leaching and determination were applied; however, since the value is defined by a technique (in contradiction to an absolutely defined value as a mass fraction), certification was not possible. The certified values for the trace elements are given in Table 11.7.

In the case of fluorine, doubts were thrown on the reliability of the oxygen bomb combustion. This method might give correct results for the analysis of some coal matrices but fusion should be systematically used in the case of fly ash; consequently,

TABLE 11.7

CERTIFIED VALUES IN CRM 038

Element	Certified value		Uncertainty		p
	(g kg ⁻¹)	(mg kg ⁻¹)	(g kg ⁻¹)	(mg kg ⁻¹)	
As		48.0		2.3	9
Cd		4.6		0.3	7
Co		53.8		1.9	9
Cu		176		9	11
Fe	33.8		0.7		12
Hg		2.10		0.15	8
Mn		479		16	13
Na	3.74		0.15		10
Pb		262		11	11
Zn		581		29	10

p: number of sets of results

the sets of laboratories using oxygen bomb combustion were discarded. In addition, destruction of fly ash by pyrohydrolysis was considered to set free the F only if a high temperature (at least 1250°C), long contact time (50–60 min), addition of reagents (e.g. V_2O_5) to attack the alkaline matrix, would be used. These precautions were a prerequisite for acceptance of the results for certification. Consequently, the methods were scrutinised and the following conclusions were drawn:

- In the case of one ISE technique, the dissolution was suspected to be insufficient. A citrate buffer was preferred to an acetate one. Moreover, it was stressed that the solution should be centrifuged prior to acidification. An alkaline filtration step allowed, however, to remove interfering cations (precipitation of Al, Fe, Ca, Mg etc.). Previous experience has shown that interfering cations could be removed if strong reagents were used which was the case. Consequently, the destruction method was accepted.
- One laboratory did not observe any difference in the results obtained with and without addition of V_2O_5 . However, the pyrohydrolysis method as used by this laboratory was suspected not to be sufficient for stripping off fluoride. It was suspected that V_2O_5 would not completely destroy the ‘Christmas tree balls’ at 1250°C; a complete fusion was assumed to be achieved at 1400–1500°C. However, at the temperature chosen the duration of the treatment and the amount of water used in the reaction became important; the volume of water was found to be too small in this case (ca. 100 mL); 500 mL should be used instead. The 20 min chosen were found to be insufficient (60 min would be more suitable). Finally, it was said that iron phosphate would have been more appropriate than V_2O_5 under the condition chosen. The sets of results were consequently withdrawn.
- The method using U_2O_8 as fuse and attacking reagent in pyrohydrolysis was verified in many round-robin exercises. In this certification, a temperature of 1250°C was applied and a volume of water of 500 mL was used for a duration of the treatment of about 60 min which was considered to be acceptable.
- A pyrohydrolysis method was applied at 1250°C for 55–60 min with addition of iron phosphate. Both the addition of iron phosphate and the long duration of the treatment were found suitable. However, the small amount of water used was suspected not to allow a complete stripping off of F, which would explain low results; this justified the withdrawal of the results.
- With regards to the final determination, the following remarks were made: the complexation of interfering cations using CDTA was not sufficient in some cases which was demonstrated by the use of stronger reagents (with ISE detection) and corresponding higher results. The amount of CDTA needed to complex metal ions should be correctly evaluated and optimised.
- A bias was detected by one laboratory in the standard addition method used (wrong slope used in the calculation): this was due to a slower conversion to the AlF^{2+} ion than expected, resulting in an incorrect slope for the calibration graphs. The analysis was repeated by allowing the samples to stand for 2 h before injection in the chromatograph which enabled to obtain better results.

Fluorine was certified on the basis of 8 sets of results (5 ISE, 1 IC, 1 FNAA and 1 SPEC). The certified value is $(538 \pm 13) \text{ mg kg}^{-1}$.

For chlorine, low results obtained with HNO_3 addition were suspected to be due to an incomplete dissolution (leaching of Cl rather than attack) which justified the rejection of one set of results. Chlorine was certified on the basis of 10 sets of results (8 IC, 1 SPEC and 1 argentometric titration). The certified value is $(323 \pm 22) \text{ mg kg}^{-1}$.

11.2.2.4. Participating laboratories

The material was prepared by the joint Research Centre of Ispra (Italy), the Department of Chemical Engineering of the University of Loughborough (United Kingdom), the Universiteit Gent, I.N.W. (Belgium), and H.G.J. Trommar Glass-Works (The Netherlands). The homogeneity and stability studies were carried out by the Centro di Radiochimica e Analisi per Attivazione in Pavia (Italy), the Universitaire Instelling Antwerpen (Belgium), Hoogovens Groep b.v. in IJmuiden (The Netherlands), and the Gesellschaft für Strahlen und Umweltforschung in Neuherberg (Germany). The following laboratories participated in the certification campaign for the trace elements: Bundesanstalt für Materialsforschungs- und Prüfung, Berlin (Germany); TNO Division of Technology for Society, Delft (The Netherlands); Centro di Radiochimica e Analisi per Attivazione, CNR, Pavia (Italy); Centro di Studi sulla Chimica Analitica Strumentale, CNR, Bari (Italy); Energieonderzoek Centrum Nederland, Petten (The Netherlands); Environmental and Medical Science Division, Harwell (United Kingdom); Hoogovens Groep b.v., IJmuiden (The Netherlands); Gemeentelijk Centraal Milieu-Laboratorium, Amsterdam (The Netherlands); Institut de Recherches de la Sidérurgie Française, Maizières-les-Metz (France); Institut für Reinst Stoff-Analyse, Gmuend (Germany); Institut für Wasser-, Boden- und Lufthygiene, Berlin (Germany); Joint Research Centre; Ispra (Italy); Joint Research Centre, Petten (The Netherlands); Kernforschungsanlage, Jülich (Germany); Kernforschungszentrum, Karlsruhe (Germany); KEMA N.V., Arnhem (The Netherlands); Laboratorio sull'Inquinamento Atmosferico, CNR, Roma (Italy); Laboratory of the Government Chemist, Teddington (United Kingdom); Service Central d'Analyse, CNRS, Vernaison (France); Studiecentrum voor Kernenergie, SCK/CEN, Mol (Belgium); Universitaire Instelling Antwerpen (Belgium); Università degli Studi, Istituto di Chimica Analitica, Rome (Italy); Universiteit Gent, I.N.W., Ghent (Belgium).

The following laboratories participated in the certification of fluorine and chlorine: Bundesanstalt für Materialsforschung- und Prüfung, Berlin (Germany); CNRS, Service Central d'Analyse, Vernaison (France); Danish Technological Institute, Hasselager (Denmark); Energieonderzoek Centrum Nederland, Petten (The Netherlands); ELSAM, Aabentaa (Denmark); ENEL, Pisa (Italy); Gordinne & Cie, Rozenburg (The Netherlands); INERIS, Verneuil-en-Halatte (France); I/S Midtkraft, Skødstrup (Denmark); KEMA N.V., Arnhem (The Netherlands); Laborelec, Linkebeek (Belgium); Ministère des Affaires Economiques, Laboratoire Central, Brussels (Belgium); Ruhrkohle AG, Essen (Germany); Stazione Sperimentale per i Combustibili, San Donato (Italy); Technical Research Centre, VTT, Espoo (Finland); TÜV, Essen (Germany); Universiteit Gent, I.N.W., Gent (Belgium); Universidad de Oviedo, Departamento de Química Física y Analítica, Oviedo (Spain); University of Plymouth, Department of Environmental Sciences, Plymouth (United Kingdom).

11.2.3. Dioxins in fly ash extract

11.2.3.1. Introduction

The origin of the dioxin problem can be traced back to the Second World War when the production of polychlorophenol-based pesticides was launched. In particular, the industrial production of 2,4,5-trichlorophenol and its corresponding herbicide 2,4,5-trichlorophenoxyacetic acid has led to a number of accidents resulting in the release of 2,3,7,8-tetrachlorodibenzo(p)dioxin into the environment. Workers exposed to the compounds released were found to suffer from an unknown skin disease caused by 2,3,7,8-tetrachlorodibenzo(p)dioxin, as identified by a German team in 1957 [18]. The occurrence of a number of accidents, one of which was the well known Seveso explosion in Italy in 1976, made the dioxin problem into what it is today: a top-priority, scientific, social, economic and political topic.

Polychlorodibenzo(p)dioxins (PCDD) and polychlorodibenzofurans (PCDF) show an extreme chemical and physical stability and are, therefore, classified as very persistent pollutants. Owing to their persistency and to their lipophilic nature, they accumulate in biota and in soils and sediments which contain a high amount of organic matter. Although there is still some controversy as to the absolute degree of toxicity of both groups of compounds, it is generally acknowledged that extreme care should be taken that tetra, penta and hexachloro compounds showing 2,3,7,8-chlorine substitution exhibit the highest toxicity.

In the last twenty years, PCDD and PCDF were identified as by-products in many industrial processes which involve chlorine or chlorinated compounds. Additionally both groups of compounds were found to be formed in a broad range of combustion processes, including accidental fires. Municipal waste incineration is particularly considered to be a very important, if not the most important, of the identified source of environmental dibenzo-p-dioxin and dibenzofuran contamination. As a consequence, the evaluation and close control of new and existing installations for their dioxin releases has become a major concern. Based on this relative importance of municipal waste incinerators, and taking into account the relative toxicity data actually available for PCDD and PCDF, it was decided to prepare and certify a crude fly ash extract (CRM 429) for the twelve more toxic PCDD and PCDF [18,19].

11.2.3.2. Preparation of the candidate reference material

About 40 kg of fly ash was collected from an average municipal waste incinerator. Three samples, taken randomly from the bulk material, were analysed, using validated laboratory procedures, in order to ensure that the levels of the twelve target congeners were adequate for certification (i.e. at least three times the limits of detection of MS). The collected material fulfilled these requirements; it was treated in separate batches of 330 g which were transferred into 2 L erlenmeyer flasks and suspended in a mixture of 1 L bidistilled water and 100 mL of 12.5 mol L⁻¹ hydrochloric acid. The suspension was stirred magnetically for 2 h. The bulk of the fly ash was separated from the supernatant after centrifugation. The precipitate was then re-suspended several times

in bidistilled water and centrifuged again until a pH-neutral supernatant was obtained. Finer particles, not recovered by centrifugation, were separated and washed until pH-neutral, by forced filtration over paper. Precipitates and residues were allowed to dry for three weeks at room temperature and in clean ambient air. The resulting lumps were ground in a mortar.

Subsequently, 450 g aliquots of the acid-treated fly ash were extracted for 46 h with toluene in three simultaneously-operating Soxhlet extractors. The extracts were combined, concentrated under a stream of ultra-pure nitrogen and the extracting solvent toluene was exchange for iso-octane. Both toluene and iso-octane were of the quality 'distilled in glass'. Blanks showed no impurities that might interfere with the final dibenzo-p-dioxin and dibenzofuran determinations. After thorough mixing and stirring, the material insoluble in iso-octane was removed by filtration over paper.

Finally, the extract was ampouled in 1.2 mL aliquots, taken from the continuously homogenized iso-octane solution, in clean brown glass ampoules which were flame-sealed. The resulting set of 1000 ampoules was stored in a refrigerator at 4°C.

As there are no reason to suspect any differentiation between different isomers of the same isomeric group during mixing and ampouling, the within- and between-ampoule homogeneity was verified by repeated group specific determinations of tetra, penta, hexa, hepta and octa-chlorodibenzo-p-dioxins and dibenzofurans. Reagents were of p.a. quality and experiments were made to assess reagent blanks. An accurately known mass of the homogenized extract (ca. 150 mg) was dissolved in 1 mL of benzene and spiked with 20 µL of an internal standard mixture [18]. The solution was placed on top of a chromatographic column (15 mm i.d.) containing 12.5 g of alumina topped with 10 g of anhydrous sulphate. The column was then washed twice, first with 50 mL of benzene and secondly with 100 mL of 2% dichloromethane in n-hexane (redistilled in glass). Both eluates were discarded. Subsequently, PCDD and PCDF were eluted using 75 mL of a 50/50 mixture of dichloromethane and n-hexane. The resulting eluate was concentrated under ultra-pure, dry nitrogen to obtain a final volume of approximately 250 µL. This concentrate was transferred to a glass tip using dichloromethane, which was afterwards substituted by n-nonane (distilled in glass). The final volume of the sample, ready for injection, was ca. 100 µL. Instrumental analysis was performed by combined gas chromatography/mass spectrometry. PCDD and PCDF concentrations were obtained from separate analyses, using the same chromatographic conditions but different mass spectrometric detection settings; the experimental conditions are described extensively in the certification report [19]. Analyses were carried out taking into account good laboratory procedure. The coefficients of variation of the results of the analysis of samples taken from the same ampoule (within-ampoule) was always less then 8.5% whereas the between-ampoules CVs were always smaller than 9%. No statistical difference was observed between the within- and between-ampoule data and the material was hence considered to be homogeneous.

The stability was verified over 12 months of storage in a refrigerator between 0 and 4°C and by comparing the results with those obtained on samples of the freshly prepared fly ash extract (12 months before). No instability could be demonstrated for any of the monitored compounds.

11.2.3.3. Certification

Each laboratory used its own procedures for the sample preparation, clean-up, method of injection, choice of carrier gas, capillary columns and other chromatographic conditions. Each participant received two calibrant solutions prepared independently and gravimetrically with PCDD and PCDF of verified purity and identify [20]; in addition, the participants received six ^{13}C labelled isomers in solution to be used as internal standards for determining the response factors and the recovery efficiency of the methods, as well as for calibration purposes [19].

The extract was cleaned up by a variety of established techniques to remove compounds that could interfere with the gas chromatographic determination of the PCDD and PCDF, either by masking the gas-chromatographic response or by degrading the performance of the instrument. The clean-up steps were either carried out using one single column or 2 to 4 successive independent columns. Clean-up techniques were based on e.g. alumina, AgNO_3 -silica gel, acid silica gel, potassium silicate, basic alumina, basic silica gel, carbon, CsOH silica gel, Florisil, anhydrous Na_2SO_4 , NaHCO_3 , Celite, H_2SO_4 , glass fibers, etc.

The PCDD and PCDF were determined by high-resolution capillary gas chromatography with mass spectrometric detection. Each isomer was identified and confirmed, using at least two columns with different stationary phases and polarity, by comparing the relative retention times of the peaks in the calibration and sample chromatograms. The final quantification was made using relative peak height or peak area. The GC and MS conditions used by each laboratory are summarised elsewhere [18,19].

Quantification was based upon the method generally referred to as internal standardisation using, within each isomeric group, one ^{13}C -labelled isomer as an internal standard. Inevitable differences in chromatographic retention and/or minor differences in ionisation efficiency, mass spectrometric fragmentation and ion masses monitored, lead to differences in sensitivities between the compounds to be determined and the corresponding ^{13}C -labelled internal standards. These effects were accounted for in the final concentration calculation by the introduction of isomer specific relative sensitivity factors (RSF). Additional details on this procedure are described elsewhere [18,19].

Due to possible differences in behaviour of the different congeners in the various steps of the clean-up procedures, the participants were requested to perform recovery experiments that included each of the individual congeners to be certified. Recovery experiments were performed by standard additions. If the recovery for the different isomers within the same isomeric group was found to be constant (within the determination uncertainty) only one recovery check was requested. In cases where the recovery varied more than the measurement uncertainty (ca. 10%), three replicate recovery estimations were requested. In case the difference between isomers was larger than the uncertainty of the measurements, the laboratory had to give evidence for the validity of the recovery estimate. Where this evidence for the validity of the clean-up was not available, the data were rejected.

The results were extensively discussed on the basis of the various technical requirements that the laboratories had to follow [19]. The following specific comments were made:

The selective determination of F83 ((2,3,7,8-T4CDF), free from interferences, requires an extremely good chromatographic resolution. Thus, all chromatographic records were screened and only those data showing sufficient separation were accepted for certification. Examples of good separation are given in the certification report [19].

The selective determination of F94 (1,2,3,7,8-P5CDF) in the fly ash extract cannot be performed on a common polar capillary column such as CP Sil 88 or SP 2331. The results obtained on these columns were not acceptable for certification. Instead a special polar column, coded DB Dioxin, performs well, as do almost all of the commonly used non-polar capillary columns (CP Sil 5 CB, DB 5, HP 5 etc.).

The results for F114 (2,3,4,7,8-P5CDF) obtained on a CB Dioxin column suffer from an unknown interference and, therefore, could not be accepted. The problem can be overcome by using a CP Sil 88 or SP 2331 phase.

Most of the chromatographic columns in use do not allow the determination of F118 (1,2,3,4,7,8-H6CDF) without interferences. A good DB Dioxin is able to separate at least some of the interferences, but column quality is very critical. Therefore, no attempts were made to certify F118 and results reported were considered as indicative only [19].

The certified PCDD and PCDF contents in the fly ash extract are given in Table 11.8, along with their uncertainties. Recommendations on the analysis of the CRM 429 are given in the certification report [19] and published elsewhere [18].

11.2.3.4. Participating laboratories

The material was prepared by the Vlaamse Instelling voor Technologisch Onderzoek (VITO) in Mol (Belgium) and the Institut des Radio-éléments (IRE) in Fleurus (Belgium); the homogeneity and stability studies were carried out by VITO. The following

TABLE 11.8

CERTIFIED PCDD AND PCDF CONTENTS IN THE FLY ASH EXTRACT

Compound	Certified value ($\mu\text{g kg}^{-1}$)	Uncertainty ($\mu\text{g kg}^{-1}$)	p
2,3,7,8 T4CDD	4.8	0.4	10
1,2,3,7,8 P5CDD	24.8	1.6	10
1,2,3,4,7,8 H6CDD	66	6	10
1,2,3,6,7,8 H6CDD	145	5	9
1,2,3,7,8,9 H6CDD	79	4	11
2,3,7,8 T4CDF	16.2	1.1	9
1,2,3,7,8 P5CDF	40.7	2.8	8
2,3,4,7,8 P5CDF	71	5	10
1,2,3,6,7,8 H6CDF	165	18	7
1,2,3,7,8,9 H6CDF	15.2	1.8	7
2,3,4,6,7,8 H6CDF	299	30	10

p: number of sets of results

laboratories participated in the certification: Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol (Belgium); Ministry of Agriculture, Fisheries and Food (MAFF), Norwich (United Kingdom); Schering Agrochemicals, Essex (United Kingdom); Warren Spring Laboratory (United Kingdom); Bayer A.G., Leverkusen (Germany); Solvay Duphar B.V., Weesp (The Netherlands); Universität Ulm (Germany); Istituto Inquinamento Atmosferico, CNR, Rome (Italy); BASF AG, Ludwigshafen (Germany); Universiteit Amsterdam (The Netherlands); Istituto Mario Negri, Milan (Italy).

11.3. DUST MATERIALS

11.3.1. Trimethyllead in urban dust

11.3.1.1. Introduction

Although the use of leaded gasoline is being increasingly discontinued in many countries, the use of tetraalkyllead compounds as antiknock agents remains the largest application of organolead compounds [21] and, owing to the ubiquity of lead and concern over the toxicity of organolead compounds in the environment [22], the monitoring of lead species will need to be continued over the next decade. Vehicular emissions of tetraalkyllead are subject to atmospheric breakdown to trialkyl- and dialkyllead and all three forms are scavenged from the atmosphere by rainfall [23]. Therefore, trimethyl- and triethyllead are found in road drainage and surface water [24]. As a consequence, a number of laboratories are performing analyses of rainwater and urban dust, for example, to monitor the levels of trialkyllead compounds in the environment. The techniques used are generally based on a combination of different analytical steps including extraction, derivatisation (e.g. ethylation or Grignard reactions), separation (e.g. gas chromatography or high performance liquid chromatography) and detection (e.g. atomic absorption or atomic emission spectrometry) which all pose risks of systematic errors (e.g. insufficient extraction, inhibition of derivatisation reaction, incomplete separation etc.). In order to verify the performance of analytical techniques used in lead speciation and the quality control of trimethyllead determinations, certified reference materials are needed. This section describes the preparation and certification of trimethyllead in an urban dust reference material, CRM 605.

11.3.1.2. Feasibility study

One of the most critical points in organometallic chemistry analysis is the availability of calibrants of suitable purity and verified stoichiometry. This aspect was recognised at an early stage of the project and the purity of alkyllead compounds used in the feasibility study was carefully verified [25]. Additional experiments were performed on calibrants in the frame of the first interlaboratory exercise. Trimethyl- (TriML) and triethyllead (TriEL) compounds were obtained from Alfa products (Johnson Matthey)

and their purity was verified as described elsewhere [26]. The two trialkyllead calibrants were not less than 98% pure.

11.3.1.3. Interlaboratory study

An interlaboratory study was carried out to test the state-of-the-art of trimethyllead determination in urban dust [27]. Urban dust was collected from the Queensway road tunnel in Birmingham city centre. After being passed through a 500 μm sieve to remove large particles of debris, the dust was first treated by air-drying for several days (4–5 days) and then ground with a ballmill for three minutes. The ground dust was further sieved through a 80 μm sieve. Around 600 g of treated road dust was homogenised thoroughly in a 1 kg glass jar and then stabilised by freeze-drying for 20 h. The material was bottled immediately into 30 mL amber glass bottles each of which contained approximately 25 g of the dust, then they were sealed into plastic bags.

In the interlaboratory study, the pretreatment techniques used were in most cases based on complexation, GC separation and employed various detection techniques (e.g. MIP-AES, AAS, ICP-MS). The extraction recovery was considered to be one of the most critical points in urban dust analysis. Direct ultrasonic extraction of TriML from the urban dust into water gave poor recoveries and in extreme cases led to negligible or even negative recoveries (in comparison to spike recovery tests); this was probably because such a treatment leads to the release of inorganic lead from the sample which consumes the reagent (NaBEt_4) despite the EDTA masking. In addition, the high amount of inorganic lead extracted creates important interferences at the detection step, which requires the addition of EDTA. Therefore, a milder extraction procedure (shaking) was used to ensure complete recovery of the TriML spike to be obtained whilst inorganic lead was only partly extracted.

Some doubts were expressed on the procedure used by one laboratory which filtered the suspension and recovered TriML from the filtrate. Indeed, it was suspected that losses could have occurred by e.g. adsorption on the filter. The extraction recovery was not necessary as standard additions were performed prior to extraction; recovery values of 66 and 77% were obtained. At this stage, it was not possible to confirm the doubts expressed over the extraction recovery of TriML in this material. The participants recommended that emphasis be put on the verification of extraction recovery in a further exercise, i.e. that a small batch of candidate reference material of urban dust be spiked with a known amount of TriML, left to equilibrate, homogenised and made available to the participants so that the extraction recovery may be verified. Recommendation was made to spike the dust in a slurry which should be freeze-dried, rather than oven-dried, in order to avoid losses of TriML.

11.3.1.4. Production of the candidate reference material

Around 15 kg of surface road dust was collected from sweeping a lay-by in the central section of the Queensway tunnel in Birmingham (UK). The tunnel is approximately 850 m in length and is a major traffic route through Birmingham from the motorway system. The dust was passed through a 500 μm sieve to remove large particles of debris.

Then, the sample was treated by air drying at room temperature for five days, on a flat tray lined with clean paper, in a well-ventilated and dark place. Further, the dust was ground in a ballmill for a period of three minutes and sieved through a 125 μm sieve.

About 10 kg of the pretreated dust sample was homogenised by the following procedure: the whole amount of dust was divided in four sub-samples of about 2.5 kg; each sub-sample was stored in a 5 L dark glass bottle and shaken on the mechanical shaker for few hours (using different shaking directions). When all four sub-samples had been homogenised, they were redistributed again into a second four sub-samples in such a way that each of the first four sub-samples contributed equally to the each of the second four sub-samples. This procedure was repeated three times after which the final four sub-samples were again combined in one sample. The homogenised dust sample was freeze-dried at -50°C and 8 millibar pressure for 24 h. The bottling procedure followed immediately. Each of the six hundred 30 mL amber glass bottles, provided with screw caps, was filled with ca. 15 g of the dust sample, sealed into a plastic bag and stored in the cold room at 4°C .

A separate batch of material was set aside for the preparation of spiked samples to be used for the verification of extraction recoveries by the certifying laboratories. A solution (250 mL) of trimethyllead chloride calibrant containing 20 μg as Pb was first prepared, and then added to 400 g of candidate urban road dust. The slurry was placed in a 1 L wide-neck amber-glass bottle and was stirred manually for several hours with two glass rods, and then stored over 48 h at 4°C . After freeze-drying over two nights, the dust was gently ground in a porcelain mortar, and then transferred into another 1 L wide-neck amber-glass bottle. The bottle was then shaken mechanically to homogenise the whole sample of the spiked road dust before it was distributed into 25 bottles, each containing ca. 15 g of the dust. The spiking level of trimethyllead in the urban road dust was $50 \mu\text{g kg}^{-1}$ (as Pb).

The within- and between-bottle homogeneity was verified at the level of 1 g with the following method: samples (1 g) were transferred to 250 mL screw cap glass bottles containing Milli-Q water (100 mL) and NaCl (10 g). The bottles were shaken on a mechanical shaker for 30 min. The slurry was filtered through two pieces of glass microfibre filter (Whatman GF/C) and rinsed with 50 mL of Milli-Q water. The combined filtrates were then transferred to a second clean 250 mL glass bottle. After the pH was adjusted to 9.0 with an ammonia solution, EDTA (3 g), 5 mL of 0.5 mol L^{-1} NaDDTC and 15 mL hexane were added. After 30 min agitation, the organic phase was removed and the extraction was repeated with a second aliquot of hexane. The combined hexane extracts were then passed through anhydrous Na_2SO_4 with hexane rinses. The hexane extracts were transferred to a 25 mL conical flask and evaporated by purging with a N_2 stream in a water bath, set at 35°C , until approximate 5 mL of the hexane extract remained. This was transferred into a 10 mL concentrator receiver tube with a hexane rinse and evaporated by purging with a N_2 stream until 0.5 mL of the extract remained. Grignard reagent (0.3 mL propylmagnesium chloride) was added and the concentrator tube was shaken in a ultrasonic bath for 5 min. 5 mL of 0.5 mol L^{-1} H_2SO_4 was added to destroy the excess Grignard reagent and the mixture was shaken in the ultrasonic bath for a further 5 min. The hexane layer was removed from above by means of a 200 μL pipette. A small amount of anhydrous Na_2SO_4 was placed within the pipette tip to

dry the extract. This 25 μL of the final extract was injected into a GC-AAS system. The extraction recovery of trimethyllead in road dust was verified by spiking road dust material at a level of 4 $\mu\text{g kg}^{-1}$. The range of recovery was from 66.5% to 91.3% for four replicates (mean of $(77.6 \pm 9.5)\%$). The results did not reveal significant differences between the within-bottle and the between-bottle variances and the method CV. On the basis of these results, no in-homogeneity was suspected and the material was considered to be homogeneous at a level of 1 g and above [28].

The stability of the material upon storage was tested in the dark at -20°C , $+20^\circ\text{C}$ and $+37^\circ\text{C}$ and trimethyllead was determined at the beginning of the storage period and after 1, 3, 6, 12 and 37 months. Samples were analysed using the same procedures as for the homogeneity study. On the basis of the results obtained, no instability of the material could be demonstrated over a period of 37 months for the material stored at $+20^\circ\text{C}$. However, a significant decrease in trimethyllead content was observed to occur at $+37^\circ\text{C}$. It was hence concluded that the material is stable at $+20^\circ\text{C}$ whereas storage temperatures above this level should be strictly avoided.

11.3.1.5. Certification

A trimethyllead calibrant was prepared by the University of Plymouth for the purpose of the certification campaign in order to enable participating laboratories to verify their own calibrants [28]. The methods used are summarised in Table 11.9.

Results produced by hydride generation/ZETAAS were withdrawn; this method is capable of speciating between inorganic and organic lead but does not have the specificity

TABLE 11.9

Summary of techniques used in the certification

Addition of $\text{NaCl}/\text{H}_2\text{O}$; Derivatisation with 4% NaBEt_4 ; Separation by cryogenic trapping in an U-tube filled with chromatographic material; Detection by QFAAS (Lab. 01)

Complexation with EDTA and DDTC; hexane extraction; Derivatisation by addition of 2 mol L^{-1} propylmagnesium chloride; elution with hexane; Separation with U-tube filled with chromatographic material; Detection by QFAAS (Lab.02)

Addition of ammonium citrate/EDTA; Derivatisation with NaBEt_4 ; Separation by CGC; detection by MIP-AES (Lab.03)

Supercritical fluid extraction using CO_2 with methanol; liquid-liquid extraction of SFE eluate with n-hexane after complexation with DDTC; Derivatisation by addition of propylmagnesium chloride; Separation by CGC; detection by MS (Lab. 04)

Addition of NaCl , EDTA and hexane; Separation by CGC; Derivatisation with NaBEt_4 ; detection with ID-ICPMS of isotopes ^{206}Pb and ^{208}Pb (Lab. 05)

Addition of DDTC; extraction with pentane; clean-up with 100% active alumina followed by elution with hexane/diethylether; Derivatisation with pentylmagnesium bromide; Separation by CGC; detection by MS (Lab. 06)

Extraction with pentane after buffering with ammonium acetate and DDTC complexation; clean-up with deactivated alumina; redissolution into hexane; Derivatisation with butylmagnesium bromide; Separation by CGC; detection by MS (Lab. 07)

to differentiate between organo-lead species. The candidate CRM was likely to contain a variety of organo-lead compounds and, therefore, this result was withdrawn.

The recoveries obtained by the various laboratories were discussed and most were in the range 70 to 95% with standard deviations of 3 to 8%. It was agreed that the best practice was to conduct recovery studies alongside the analytical measurement. Trimethyllead in the spiked sample was determined as the difference between the spiked and the unspiked results.

The certified value and its uncertainty (after both technical and statistical scrutiny) is $(7.9 \pm 1.2) \mu\text{g kg}^{-1}$. Trimethyllead is certified as mass fractions of $(\text{CH}_3)_3\text{Pb}^+$ ($\mu\text{g kg}^{-1}$ as TriML).

11.3.1.6. Participating laboratories

The certification campaign has been coordinated by the Department of Environmental Sciences of the University of Plymouth (UK). The sample preparation, homogeneity and stability studies were carried out by the School of Chemistry of the University of Birmingham (UK). The analyses for certification were performed by the following laboratories: De Montfort University, Department of Chemistry, Leicester (United Kingdom); Institut für Chemo- und Biosensorik, Münster (Germany); Universitaire Instelling Antwerpen, Departement Scheikunde, Wilrijk (Belgium); University of Birmingham, School of Chemistry, Birmingham (United Kingdom); University of Plymouth, Department of Environmental Sciences, Plymouth (United Kingdom); University of Umeå, Department of Chemistry, Umeå (Sweden); Universidad de Zaragoza, Centro Politécnico Superior de Ingenieros, Zaragoza (Spain); Vrije Universiteit Amsterdam, Instituut voor Milieuvraagstukken, Amsterdam (The Netherlands).

11.3.2. Cr(VI) in welding dust

11.3.2.1. Introduction

The different toxicity and bioavailability of Cr(III) and Cr(VI) are a public health concern and therefore require strict control. Trivalent chromium is found to be essential for man where it is involved in glucose, lipid and protein metabolism, whereas the deleterious effects to living organisms of Cr(VI) are well documented. Cr(VI) is also a potent carcinogenic agent for the respiratory tract requiring continuous monitoring of occupational air. Hence monitoring of the separate species in *e.g.* drinking water, occupational exposure or environmental samples is necessary. Determination of the total Cr content does not provide sufficient information about possible health hazards. Hexavalent chromium is such a potent carcinogenic agent for the respiratory tract that continuous monitoring is imposed, stated in the Directive 90/3941/EEC on exposure to carcinogenic substances. In occupational health, the OEL (Occupational Exposure Limits) for water soluble and water insoluble compounds in indoor air is limited to 0.5 mg m^{-3} for chromium, to 0.5 mg m^{-3} for Cr(III) and to 0.05 mg m^{-3} for Cr(VI) which reflects the different toxicity of both species.

In order to meet the requirements of the Directive and OEL standards, the reliability

of the methods needs to be improved substantially and appropriate reference materials certified for Cr(III) and Cr(VI) are hence needed. This section describes the preparation and certification of Cr(VI) and total leachable Cr in welding dust loaded on a filter as normally encountered on the personal filter monitors of stainless steel welders, CRM 545 [29].

11.3.2.2. Preliminary investigations

A feasibility study was carried out to develop and optimise the methodology to produce a batch of filters with a homogeneous and stable distribution of Cr(VI) and a load of Cr(VI) corresponding to what is usually found on occupationally charged filters [30]. The particle size distribution of the welding fumes determinates the degree to which fumes may be inhaled and how they are deposited within the respiratory tract. The particle size distribution was therefore investigated. Scanning transmission electron microscopy of the welding fumes shows an agglomerate of the fume particles. The particles, which tend to form chains, have a diameter of approximately 0.2 μm and are alkalichromates and silicates of Na, K, Mn, Fe, Ni and Cu. Energy dispersive spectroscopy (EDS) has been used to give qualitative elemental analysis, indicating possible inhomogeneous distribution in the agglomerate for minor components since Ni was not found in the chosen particle although there is 0.6% Ni in the electrode used to produce the welding fumes.

An intercomparison on European scale was organised in 1994. It was designed to identify pitfalls and sources of error and consequently to improve the skills of many laboratories to such a degree that certification of a reference material became feasible. The intercomparison would also prove the suitability of the materials produced. For this purpose, two batches of 100 filters each, loaded with welding dust, containing approximately 100 μg Cr(VI), were made. The procedure described by S. Dyg et al. [30] was followed in general. To detect analytical problems not related to leaching of the filters, the participants were asked to analyse a lyophilised solution, containing 5–10 mg L^{-1} Cr(VI) as well. The results of the intercomparison were scrutinised on scientific grounds during a meeting with all the participants [31].

One of the parameters that might influence the results for the Cr(VI) content in welding dust is the leaching method. Leaching at low pH could cause reduction of Cr(VI) due to Fe(II) or organic substances leached as well. Leaching at high pH in the presence of oxygen on the other hand might lead to oxidation of Cr(III) yielding too high results for Cr(VI). Most of the laboratories used the NIOSH method (2% NaOH-3% Na_2CO_3) [32]; other methods were based on e.g. NaHCO_3 buffer at pH 6.4, NaOAc buffer at pH 4, pyridine carboxylic acid buffer or H_2SO_4 . Some laboratories found Cr(VI) values in the filters that exceeded the Instrumental Neutron Activation Analysis results for total Cr in the dust (39.3 ± 1.3 g kg^{-1}) obtained by the coordinating laboratory and the results submitted were consequently withdrawn. The calculated mean of means resulting from 12 laboratories was (34.5 ± 1.5) g kg^{-1} Cr(VI) in the dust, corresponding to a coefficient of variation of 4.3%. The outcome of this intercomparison was promising enough to justify the certification of identical filters with similar concentrations.

11.3.2.3. Production of the candidate reference material

A total of 1100 filters, loaded with welding dust containing approximately 100 µg of Cr(VI) was produced in 1994 [30]. Prior to dust collection, the filters (borosilicate microfibre glass discs without resin binder) were acclimatised in an air-conditioned laboratory (22°C and 50% relative humidity) for 24 h, and weighed carefully together with the two PTFE rings intended to avoid losses of glass fibre on the monitor when pressed together. The monitors were assembled and mounted in the Sputnik air-sampling unit. The Sputnik air sampler consists of a round vacuum chamber of stainless steel in which 100 critical orifices (Ø 0.4 mm) are placed. They are positioned in 4 rings containing 36, 29, 22 and 13 orifices respectively. Two vacuum pumps secure a constant vacuum of 0.66 bar in the pressure chamber, which on its turn secures a homogeneous airflow of 1.9 L min⁻¹ through each orifice and filter. A pilot study demonstrated that the airflow through the inlet of the monitor mounted on the critical orifice was not disturbed or changed neither by different types of filters placed in the monitor nor by the number of filters mounted on the manifold or critical orifice plugged by a rubber tube.

Loading was done with welding fume dust originating from a manual metal arc (MMA) computerised welding system, using an electrode which produces a welding fume dust containing approximately 4.3% Cr(VI). Fume analysis by the manufacturer showed following composition; Fe: 6.5%; Mn: 3.1%; F: 16.5%; Pb: 0.06%; Cu: 0.02%; Ni: 0.66%; Cr(III): 1.2% and Cr(VI): 4.3%. Loading was done in 12 batches of 100 filters each. All batches were made on the same day by the same operator to guarantee reproducibility. To avoid contamination, the welding was performed in a special fume box.

After charging, the Sputnik was flushed with Ar for 5 min while the pumps were still on. A second weighing of the filters took place following 24 h acclimatisation in an air conditioned laboratory. The monitors were re-assembled and flushed with N₂ to assure storage of the filters in an inert atmosphere. They were finally sealed with the end caps, labelled and numbered. The filters were stored in the dark at room temperature.

To check the between-filter homogeneity, 40 filters were randomly set aside after loading them with welding dust. They were taken from the different batches. The filter and PTFE rings were placed in a 25 mL beaker with the dust loaded side facing upwards. The filter was covered with 10 mL of an alkaline buffer (2% NaOH–3% Na₂CO₃) and a small conical PTFE ring was placed on top to secure the filter beneath the surface of the liquid. This assembly was covered with laboratory film and subjected to agitation in a heated (70°C) ultrasonic bath for 30 min. Before separation and analysis the supernatant was diluted 1000 times with a HCO₃⁻/H₂CO₃ buffer pH 6.4 to reach the linear range in ETAAS. The separation of Cr species took place immediately after leaching of the sample. The separation method is based on the cationic behaviour of Cr(III) and the anionic behaviour of Cr(VI) species. It relies on an ion exchange extraction using the liquid anion exchanger Amberlite LA-2. The liquid anion exchange solution (LAES) is obtained by stripping the Amberlite in HCl (6 mol L⁻¹) and diluting it in methyl isobutyl ketone (MIBK) (2:1:2). Cr(VI) is extracted completely into the organic phase while Cr(III) remains in the aqueous phase. To check the selectivity of the separation method, 3 blank filters were spiked with 100 µg Cr(III) and subjected to the

analytical procedure. The Cr content in the aqueous fraction after extraction was always below the detection limit of ETAAS indicating a complete precipitation of Cr(III) in the initial alkaline leaching. Care was taken to perform the measurements in the most repeatable way. The methodological uncertainty was determined by five replicate analyses of one reconstituted sample. No significant difference was found between the CV for Cr(VI) in filters and the CV for the analytical method. It could therefore be concluded that Cr(VI) is distributed homogeneously over the different filters.

Filters were kept at respectively +5°C and +20°C over a period of 12 months, and the Cr(VI) and total soluble Cr contents were determined at regular intervals during the storage period. An additional stability test was performed after 29 months, consisting in the determination of Cr(VI) at +20°C followed by a short term stability test at +40°C to simulate worst-case transport conditions. Tests were made after 1 week, 1, 6 and 12 months. Samples were analysed using the same procedure as for the homogeneity study. Cr(VI) and total soluble Cr were each determined in three fold (one replicate analysis in each of 3 filters stored at different temperatures) at each occasion of analysis. No instability could be found at any of the temperatures tested; even storage at +40°C for 14 days does not likely affect the samples.

11.3.2.4. Certification

The methods are summarised in Table 11.10. The leaching method used was generally carried out with NaOH and Na₂CO₃ buffer and agitation in heated ultrasonic bath.

The total Cr content of the welding dust, including metallic Cr, Cr(III) etc. was determined by INAA and was $(49.57 \pm 2.79) \text{ g kg}^{-1}$ dust.

Two laboratories, using respectively SPEC and ETAAS as final detection, leached the filters with H₂SO₄. Since leaching at low pH can cause reduction of Cr(VI) by Fe(II) or organic substances, those results were suspected and were withdrawn.

The results accepted after both technical and statistical scrutiny were used for the calculation of the certified values which were based on 11 sets of results in the case of Cr(VI) for a certified content of $(40.2 \pm 0.6) \text{ g kg}^{-1}$ dust and on 7 sets of results in the case of total leachable Cr for a certified content of $(39.5 \pm 1.3) \text{ g kg}^{-1}$ dust. The fact that the total leachable Cr content of the welding dust is somewhat lower than the Cr(VI) content is only due to the uncertainty on the results used for calculation of the certified values.

11.3.2.5. Participating laboratories

The certification was coordinated by the Universiteit Gent (Belgium). The sample preparation, homogeneity and stability studies were performed by the Department of Chemistry and Biochemistry of the Institute for Occupational Health (Arbejdsmiljøinstituttet) in Copenhagen (Denmark). The following laboratories participated in the certification campaign: Arbejdsmiljøinstituttet/ Dept. of Chemistry and Biochemistry, Copenhagen (Denmark); Bayer Antwerpen, Centraal Analytisch Labo, Antwerp (Belgium); Berufsgenossenschaftliches Institut für Arbeitssicherheit, Sankt Augustin (Germany); Institut für Gefahrstoff-Forschung, Bochum (Germany); Institut Pasteur,

TABLE 11.10

SUMMARY OF THE TECHNIQUES USED IN THE CERTIFICATION. THE LEACHING METHOD USED WAS GENERALLY CARRIED OUT WITH NaOH AND Na_2CO_3 BUFFER AND AGITATION IN HEATED ULTRASONIC BATH

Summary of techniques

Dilution with ($\text{NaOH}/\text{Na}_2\text{CO}_3$) buffer; dilution with $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer; filtration over $0.45\ \mu\text{m}$; ion chromatography; post-column reaction with DPC solution; detection by SPEC with DPC for Cr(VI) and ETAAS for total leachable Cr (Lab. 01)

Dilution with $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer; extraction with liquid anion exchange solution (Amberlite LA-2/MIBK); detection by ETAAS for Cr(VI) and ICP-AES for total leachable Cr (Lab. 02)

Dilution with Na_2CO_3 buffer; ion chromatography; detection by UV-SPEC (Lab. 10)

Dilution with $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer; addition of known amount of $^{53}\text{Cr(III)}$ - $^{53}\text{Cr(VI)}$ enriched spike; extraction with liquid anion exchange solution (Amberlite LA-2/MIBK); back-extraction of Cr(VI) with ammonia solution; electrodeposition on Pt wire in ammonia solution; detection by IDMS of masses 52 and 53 (Lab. 11)

Filtration at $0.2\ \mu\text{m}$; addition of HNO_3 ; detection by FAAS (Lab. 15)

Dilution with H_2O ; detection by ICP-AES (Lab. 16)

Filtration at $0.45\ \mu\text{m}$; ion chromatography; post-column reaction with methanol; detection by SPEC with DPC (Lab. 25)

Dilution with $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer; extraction with liquid anion exchange solution (Amberlite LA-2/MIBK); detection by ETAAS (Lab. 26)

Dilution with $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer; extraction with liquid anion exchange solution (Amberlite LA-2/MIBK); detection by ETAAS (Lab. 27)

Dilution with $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer; extraction with liquid anion exchange solution (Amberlite LA-2/MIBK); detection by ETAAS (Lab. 28)

Service Eaux et Environnement, Lille (France); Institute of Occupational Health, Oulu (Finland); Universiteit Gent, Laboratorium voor Analytische Scheikunde, Gent (Belgium); National Institute of Occupational Health, Oslo (Norway); Universität Regensburg, Institut für Anorganische Chemie, Regensburg (Germany); VITO, Mol (Belgium).

11.4. CBs IN WASTE MINERAL OIL

11.4.1. Introduction

Unwanted polychlorinated biphenyl (PCB) formulations and waste materials containing PCBs are normally destroyed by high temperature incineration. However, this is a relatively costly method of waste disposal and alternative, but illegal, practices are known to occur, such as dissolving PCBs in waste mineral oil. Unless the oil is chemically analysed for the presence of the CBs it will pass as waste oil which can be

buried in landfill sites or in containers at sea, leaving the persistent and toxic CBs to eventually leach out and contaminate the natural environment, or the waste oil is used for heating purposes at relatively low temperature where CBs can easily be converted into 'dioxins'.

This section describes the preparation of two mineral oil reference materials representative of many of the waste oils containing a low level ($<5 \text{ mg kg}^{-1}$) and a high level ($1\text{--}50 \text{ mg kg}^{-1}$) of total CBs which are analysed on a regular basis by a wide number of analytical laboratories. Five CBs have been certified in the low mineral oil (CRM 420) and nine CBs have been certified in the high level oil (CRM 449) [33].

11.4.2. Production of the reference materials

Low level oil (CRM 420): The waste mineral oil was obtained from a disposal site in Bergen, Norway and shipped to the test laboratory in Aberdeen, United Kingdom, in a 35 L plastic barrel. The barrel stood for two months to give any larger particles time to settle to the bottom. Twelve litres of the oil were siphoned from the upper half of the contents of the barrel into a clean, glass aspirator and mixed. The oil was filtered under vacuum through a 10 micron membrane filter to remove small metal particles and the larger carbon particles, and mixed again. The material was ampouled following a preliminary analysis to determine the water content and the approximate concentration of the CBs in the mineral oil. The oil was continuously stirred for 24 h prior to dispensing and filling of the brown glass ampoules up to the conclusion of this activity. The filling was done in charges of 50 ampoules. Each ampoule was flushed with argon, then it was filled with about 7.5 g of oil and flame sealed. Care was taken that the neck of the ampoule was not contaminated by the oil. This procedure was repeated 22 times until 1100 ampoules had been filled and sealed. All ampoules have been stored in the dark at 5°C.

High level oil (CRM 449): The waste mineral oil containing a high level of CBs (ca. 300 mg kg^{-1}) was not readily available and, therefore, it was necessary to prepare this material by doping with a chlorobiphenyl formulation. The viscous mineral oil selected was a recycled oil which had been treated by industrial centrifugation to remove particulate and water. This oil was then spiked with a transformer oil containing Aroclor 1254. After addition of this transformer oil, the mixture was thoroughly homogenised for 4 h by mixing in a drum prior to dispensing into clean Duran 50 amber glass ampoules. Each ampoule was flushed with argon prior to filling and during the heat sealing process. All the ampoules were stored in the dark at 5°C prior to dispatch.

The within- and between-ampoule homogeneity was verified by repeated determination of the seven selected CBs. A sample of the homogenised oil (200 mg) was dissolved in 2 mL of hexane and pipetted onto a chromatographic column (6.2 mm i.d.) containing 3 g of alumina impregnated with potassium hydroxide. The sample was eluted with hexane; the first 15 mL were collected. This fraction was concentrated by evaporation in a clean, dry stream of air and then pipetted onto a second column containing 3 g of silica gel. The column packing was made by heating silica gel at 150°C for 4 h, cooling it to 30–40°C, then mixing with distilled water. Hexane was the elution of which the first 5 mL were discarded and the following 15 mL were collected for further analysis.

The solution was again concentrated to 1 mL in a clean, dry stream of air, diluted to 5 mL with iso-octane and concentrated to 2 mL. Approximately 1 mL was weighed out and after addition of the internal standards, dichlorobenzyl, hexyl and hexadecyl ethers (0.39 g of a 1 mg kg⁻¹ solution in iso-octane), transferred to a gas chromatograph (GC) autosample vial prior to the final CB determinations. This solution was injected (0.5 µL) onto a 50 m fused silica capillary GC column of which the conditions are described elsewhere [33]. The calibrants and blanks were treated in exactly the same way as the samples. The recoveries of the CBs varied between 80% and 100%. The linearity of the ECD detector was tested with three calibrant solutions of different concentrations. The CV values for the determination of the CBs taken from separate ampoules were less than 9% for CRM 420 and less than 5% for CRM 449. There was no significant difference in values of within-ampoule and between-ampoule variances.

The stability was tested at -20°C, +20°C and +40°C over a period of 12 months, using the same method that in the homogeneity study. No instability could be demonstrated for any of the certified CBs.

11.4.3. Certification

The method of analysis for the final determination of the seven CB congeners used by each participating laboratory was based on capillary gas chromatography with electron capture detection. In addition, mass spectrometry was used for compound identification and confirmation, but not for quantification. Each laboratory used their own proven procedures for the sample preparation, clean-up, method of injection, choice of carrier gas and chromatographic condition. The mineral oils were dissolved in an appropriate solvent and analysed without any preliminary extraction from the matrix. The clean-up and the final determination were completed within the normal time scale for these analyses.

A set of calibrants containing the seven CB congeners was supplied to each participating laboratory in the form of pure, crystalline, certified materials from BCR (CRMs 291–298). Each laboratory was requested to prepare separate calibration solutions of the appropriate concentration in iso-octane, to determine the linearity of the electron capture detector and to calibrate the gas chromatograph prior to the analysis of the mineral oils.

The choice of internal standards was left to the participants. However, a set of pure DCBEs (dichloro-benzyl-alkyl-ethers) was made available to each laboratory by the Scottish Office Agriculture and Fisheries Department in Aberdeen for use with the calibrants provided and the samples.

A detailed description of the methods used is given in the certification report [33], including the internal standards used, GC conditions, calibration techniques etc. Clean-up procedures were based on well proven techniques to remove the bulk of the mineral oil and other organic compounds which would interfere with the chromatographic determination, e.g. decomposition with concentrated H₂SO₄, adsorption chromatography, separation on silica gel, or separation with Florisil or potassium silicate. For the separation, each CB was identified and confirmed using at least two capillary columns coated with different stationary phases to compare the relative retention times or

retention indices of the peaks from calibrants with the peaks from the waste mineral oil chromatograms. The extraction recovery was determined by spiking the mineral oil with a known mass of each congener at 4 different levels and comparing the expected and measured values; the values ranged from ca. 80% to ca. 110%.

CB 52 was not certified because there was insufficient agreement between the participating laboratories.

Difficulties were experienced for some laboratories to achieve a proper chromatographic resolution for the separation of CB 101 from CB 84 (2,3,6,2',3-pentachlorobiphenyl) and CB 118 from CB 149 (2,4,5,2',3',6-hexachlorobiphenyl); only results from those laboratories which presented chromatograms which clearly indicated a good separation were accepted for certification.

CB 138 cannot be resolved from CB 163 on most common chromatographic column phases. It requires negative-ion chemical ionisation mass spectrometry to confirm the presence of both congeners and a very polar column (e.g. SP 2331) to separate them. One laboratory was able to determine both CB 138 and CB 163 using this phase. However, using this type of column, it is possible that other CBs may also interfere with CB 138 as the retention indices for all congeners on these columns are not known. Another laboratory was able to make an HPLC separation of CB 138 and CB 163 using a 2-(1-pyrenyl) ethyldimethyl silylated silica column which is able to resolve planar and non-planar molecules. Since it has been confirmed that this oil contains both CB 138 and CB 163 and that most participating laboratories were unable to make a chromatographic separation between these congeners, the data for CB 138 + CB 163 were given as indicative values.

CB 149 was not certified because there was not sufficient agreement between the participating laboratories. High results were suspected to be due to the potential overlap between CB 118, which closely elutes with CB 149 on some GC phases.

Additional analysis by GC-MS was performed on the CRM 420 as a confirmation of the identity of the compounds and to detect any compound with the same retention index as CB 28, CB 52, CB 101, CB 105, CB 118, CB 153, CB 156, CB 170 and CB 180 [33].

The certified values of the CBs in both CRMs are given in Table 11.11, along with their uncertainties.

11.4.4. Participating laboratories

The CRM 420 was prepared by the Department of Agriculture and Fisheries for Scotland in Aberdeen (United Kingdom) whereas the CRM 449 was prepared by SGS-Depauw and Stokoe n.v. EcoCare in Antwerpen (Belgium).

The following laboratories participated in the certification of CRM 420: Department of Agriculture and Fisheries for Scotland, Aberdeen (United Kingdom); Direção Geral da Qualidade do Ambiente, Lisbon (Portugal); Institut Quimic de Sarria, Barcelona (Spain); Institute of Marine Research, Bergen (Norway); Instituto Hidrográfico, Lisbon (Portugal); Joint Research Centre, Ispra (Italy); Laboratoire Municipal, Rouen (France); National Swedish Environmental Protection Board, Solna (Sweden); Netherlands Institute for Fishery Investigations, Ijmuiden (The Netherlands); Studiecentrum für

TABLE 11.11

CERTIFIED CB CONTENTS IN CRM 420 AND CRM 449

Compound (CB IUPAC Nr)	CRM 420 Certified value \pm uncertainty (mg kg ⁻¹)	CRM 449 Certified value \pm uncertainty (mg kg ⁻¹)
28	0.61 \pm 0.06	0.80 \pm 0.07
52	not certified	31.4 \pm 1.8
101	1.45 \pm 18	57.2 \pm 1.9
105	not certified	17.4 \pm 1.0
118	1.69 \pm 0.14	46.6 \pm 2.4
128	not certified	12.5 \pm 0.7
153	0.92 \pm 0.06	39.0 \pm 1.7
156	not certified	6.9 \pm 0.5
170	not certified	6.6 \pm 0.5
180	0.195 \pm 0.017	10.4 \pm 0.5

Kernernergie (SCK/CEN), Mol (Belgium); Vrije Universiteit Amsterdam (The Netherlands).

The following laboratories participated in the certification of CRM 449: Bureau de Recherche Géologique et Minière (BRGM), Orléans (France); CID-CSCIC, Departamento de Química Ambiental, Barcelona (Spain); Department of Agriculture and Fisheries for Scotland, Aberdeen (United Kingdom); Direcção Geral da Qualidade do Ambiente, Lisbon (Portugal); Elf-Atochem, Levallois Perret (France); EMPA, Dubendorf (Switzerland); Institut Quimic de Sarria, Barcelona (Spain); Institut du Génie de l'Environnement, Lausanne (Switzerland); Institute of Marine Research, Bergen (Norway); Joint Research Centre, Ispra (Italy); Laboratoire Cantonal, Genève (Switzerland); Laboratoire Municipal, Rouen (France); National Swedish Environmental Protection Board, Solna (Sweden); Netherlands Institute for Fishery Investigations, IJmuiden (The Netherlands); S.G.S. Depauw & Stokoe n.v. EcoCare, Antwerpen (Belgium); Universidad de Barcelona, Departamento de Química Analítica, Barcelona (Spain); Universität Ulm, Abteilung Analytische Chemie, Ulm (Germany); Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol (Belgium); Vrije Universiteit Amsterdam (The Netherlands).

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Chapter 12

Interlaboratory studies

12.1. INTRODUCTION

The best way to make progress in a particular field is to collaborate with colleagues who work in similar areas of analytical sciences. By sharing expertise, many difficulties encountered in the laboratory can be solved and improvement can be obtained. The dissemination of information through literature, workshops or international conferences, and the exchange of ideas during meetings, is important but is not sufficient to improve the state of the art and maintain the quality of work in a given analytical field. Therefore, beside the transfer of knowledge via the 'classical' routes, many analysts have, for a long time, already started to exchange samples, calibrants, reference materials, in order to test their techniques. The BCR has conducted series of interlaboratory studies aiming at evaluating and possibly improving and validating analytical methods, laboratory performance or certify reference materials. The present chapter describes the principles applied in the interlaboratory studies as followed by BCR and gives several examples.

12.2. DEFINITIONS

Interlaboratory studies play an important role in the improvement of the quality of determinations. They can be applied as:

- method performance studies
- laboratory performance studies or proficiency testing;
- material certification studies.

The definitions of the various types of interlaboratory studies have been given by W. Horwitz in an IUPAC report [1] and are detailed in the following sections. All these interlaboratory studies have different goals and present in their organisation different particular aspects but they also have many items in common. The general descriptions are issued from IUPAC/ISO protocols [2,3] and recent author's contributions [4,5].

12.3. GENERAL PRINCIPLES

IUPAC has issued a 'Protocol for the design and interpretation of collaborative studies' [2] for method performance studies and 'the international harmonised protocol for the proficiency testing of (chemical) analytical laboratories' [3]. These protocols list

and discuss several items for the organisation of interlaboratory studies. The present chapter intends to detail some aspects of the IUPAC/ISO protocols in particular with respect to micro- and trace-analysis from which the various examples illustrating the general principles are taken. Additional information on the production of test materials can be found in the ISO Guide 35 on the certification of reference materials [6] and in the chapter on reference materials of this book.

All types of interlaboratory studies have the three following items in common:

- several laboratories participate;
- a common material is analysed;
- an organiser distributes the material, collects and evaluates the data and possibly organises a discussion of the results.

A trial organised between some laboratories in a private, spontaneous manner and without repetitive aspect will not be considered as a real interlaboratory study. Interlaboratory studies as such are exercises, which are planned (and discussed) beforehand, and the objective has to be well understood.

12.3.1. Participants

Achieving or maintaining a good quality requires motivation. Participants must believe that a high standard of working is achievable in their respective laboratories. Participation in interlaboratory studies may also be mandatory e.g. as a prerequisite for accreditation.

In the IUPAC 'Nomenclature For Interlaboratory Studies' [1], W. Horwitz stresses that at least eight set of data are considered to be a minimum for a sound statistical treatment of the data to test the performance of a method. The organisation, interpretation of the data etc depends on the number of participants and the objective of the study. In fact this number may vary from some few to hundreds of laboratories.

Before participating in interlaboratory studies the laboratory must have set-up all adequate internal quality assurance and quality control systems [7]. This also means that all basic investigations have been performed for possible mistakes, that these have been noticed and corrected. In other words: the laboratory has validated the method to be applied. For a laboratory performance study, this also implies that in the laboratory the method is under statistical control for a given type of matrix, that this control is monitored and that results are evaluated.

12.3.2. Organiser

The organiser of an interlaboratory study must take into account the objective of the exercise. There is a large difference of responsibility and work involved between a simple exercise on major elements in alloys for a method performance study involving some laboratories and the certification of traces of organic contaminants in a matrix material. But in all cases the organiser has to fulfil the following tasks.

- supply of clear information to the participants;
- production and distribution of the samples;
- giving evidence that the samples are adequate for the aim of the exercise

(representativeness for the analytical problem studied, stability, homogeneity etc);

- collection of results;
- evaluation of the collected data and possibly organisation of a meeting with the participants to discuss them;
- presentation of the results;
- statistical treatment of the results on which no doubt can be shed on technical grounds.

The organiser may only be the manager of the study and may delegate the technical items to adequate partners. For interlaboratory studies either leading to decisions which affect the professional status of the participants (e.g. proficiency testing within accreditation systems), or leading to certification of materials, the study manager should be evaluated [8]. In such cases the organiser has to be independent from the group of participants. His selection should even be subject to well-established rules or procedures, which could be those, set up in an EN 45000 or ISO 9000 type of norm. If confidentiality of data and of laboratory coding is required it should be guaranteed. The outcome and acceptance of an interlaboratory study will depend on the degree of confidence of the participants.

12.3.3. Objectives of the study

Another determining factor for success is the clarity and transparency of the objectives. The participants must clearly know whether the goal is to validate a well-defined method, to evaluate the laboratory (e.g. in a proficiency testing) or if the exercise is supposed to lead to the certification of a reference material.

The information on the material(s) to be analysed, the planning of the work, the exact analytical task requested and the deadline(s) to be respected should be clear. Adequate protocols should be prepared to ensure that the collected data can be compared and adequately processed.

12.3.4. Choice and preparation of test materials

12.3.4.1. Selection of the test material

All participants should receive identical test samples in order to make results as obtained by the laboratories comparable. The material prepared for the exercise should meet requirements set by the objective of the study. Basic quality requirements for reference materials used in interlaboratory studies are described in chapter 4 of this book and the reader should refer to this chapter for matters related to the choice and representativeness of the test materials. The material prepared should be homogeneous; its stability should be guaranteed at least over the duration of the study.

12.3.4.2. Representativeness

The measures to be taken to guarantee the homogeneity and the stability of the test samples may affect the representativeness of the material and obligation to compromise.

Certain parameters are impossible to stabilise, even for very short periods so that the organisation of an interlaboratory study is in fact impossible. This is the case of volatile compounds in their original matrix e.g. halogenated volatile hydrocarbons or gases in contaminated soils etc, or highly unstable compounds e.g. free radicals, nitrosamines etc. In such cases the interlaboratory studies cannot be conducted by transferring simple test samples between laboratories but may be organised in a central place where each participant applies his own technique. Such studies are only possible when the analytical methods to be applied use simple equipment e.g. glassware, burettes, pipettes etc, hardly when heavy equipment is involved such as atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP), chromatographic techniques or mass spectrometry (MS) etc. It is a well-adapted approach for testing e.g. microbiological methods. Meeting in a central place is also a sound alternative when the interlaboratory study focuses on sampling techniques. Another alternative would be to prepare a kind of intermediate or a compromise sample which is stable e.g. volatile substances trapped on adsorbents in closed systems, a substance separated from its normal matrix which induces the instability such as organo-phosphorus or organo-nitrogen pesticides in water [9].

12.3.4.3. Preparation

When the matrix has been chosen with regards to the feasibility as discussed above and when the parameters to be measured are clearly defined, the collection and the preparation of the test material may start. All requirements of quality, except the certification of the content of certain substances, are valid for all type of reference materials used in interlaboratory studies. These detailed requirements, the preparation and control procedures should be available from the organiser in a production report. They are discussed in more detail in the chapter 4 dealing with (C)RM.

12.3.4.4. Assigned values

The interlaboratory study should possibly lead to conclusions useful to the participants. Therefore, the results of the exercise have to be made available to them. To arrive at clear conclusions it is not sufficient to know only the performance of other participants, the mean value of the means and the between laboratory standard deviation. In fact the conclusions which can be drawn may be subject to ambiguity, especially with regards to trueness. If one biased method is used by a majority of participants of a group one individual laboratory using a more accurate method may have an outlying value. Figure 12.1 shows the results of an interlaboratory exercise on the determination of Sn in brass; laboratories which applied 'wet methods' with an acidic dissolution were all confronted to losses due to precipitation (the mean is far from the target value calculated from the preparation procedure).

The same may happen if a step in a method remains ambiguous and is systematically applied by a majority of analysts. The role of interlaboratory studies is also to be a way to help laboratories and methods to achieve accuracy, it is important to know the 'true value' to be found and a reliable uncertainty of this value. However, often this

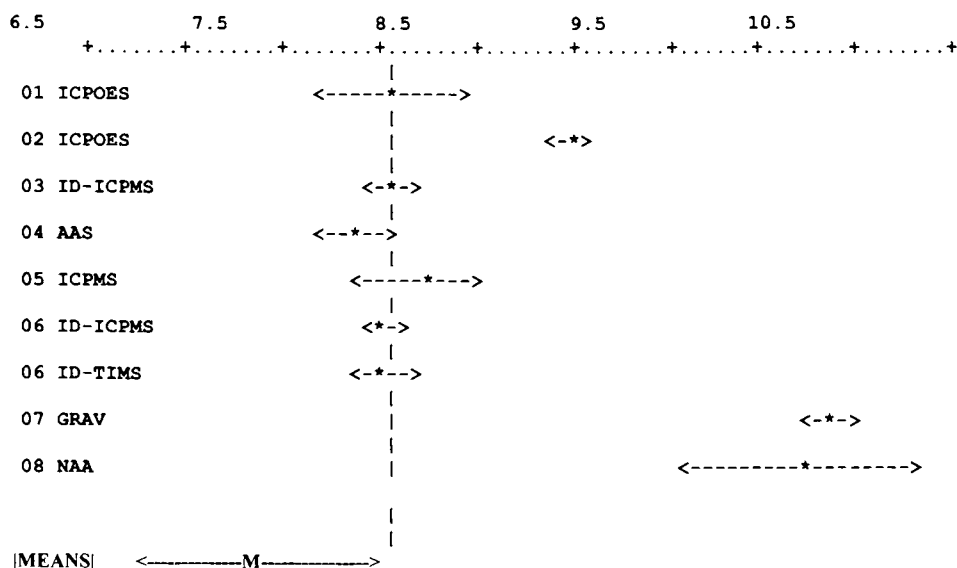


Fig. 12.1. Bar-graph of tin in archaeological brass material (Cu matrix containing: Sn 10%, Pb 10%, As 0.3%, Zn 0.10%, Fe 0.10%, Mn 0.10%, Ni 0.30% and Sb 0.30%).

The results correspond to six replicate determinations. 'M' is the mean of laboratory means with standard deviation. The theoretical content based on the production process is 10% and can be considered as a kind of target value. Several laboratories applied spectrometric methods, which needed dissolution of the sample; all were faced with a slight precipitation of tin oxide without noticing it in the strong coloured solutions. They lost about 20% of Sn. Even isotope dilution techniques (Thermal Ionisation or ICP MS) could not circumvent the problem as the spikes were not in oxidised form. Neutron activation did not need any pretreatment. Therefore, the result confirms the theoretical value of 10%. Gravimetry was faced with a co-precipitation of Sb, which explains the value higher than the target. In such a case, without target value, the interlaboratory study could have led to the conclusion that the NAA results were wrong.

value cannot be known or is difficult to approach. The assigned value may in certain cases be the calculated concentration of an element or compound in a solution when the measured masses have been produced in metrologically valid conditions with compounds of verified composition (identity, stoichiometry) and purity. Apart from aqueous or organic artificial solutions or mixtures of pure compounds all natural solid or liquid materials (matrix materials) can only be characterised by an analytical procedure. However, as it has been stressed above it may be misleading to deduce the assigned value and the associated uncertainty from the simple calculation of the between laboratory mean and standard deviation. It is the responsibility of the organising body to select and control the laboratories in charge of the determination of this assigned value. Full trueness can only be approached by applying the same rules as for CRMs. Nevertheless, experienced laboratories can prepare reliable samples and establish an assigned value with few colleagues before launching interlaboratory studies.

CRMs should not be used in large collaborative laboratory performance studies.

They would guarantee the best accuracy possible but participating laboratories could recognise them.

12.3.5. Analytical protocol

An interlaboratory exercise is organised with a defined task. The minimum information, which has to be given in the protocol accompanying the test samples, is the specification of the task of the study.

The protocol should specify at least the number of test samples, the number of replicates to be performed, and some practical instructions on receipt, preservation of the samples, sub-sampling, sample pretreatment, and on the reporting of the data. In practice this means that the requirements which have to be specified in the analytical protocol have to highlight the exact analytical task to be performed.

An example may be found in the determination of pesticides in a milk powder: laboratories monitoring the quality of milk products usually express the data on the basis of the fat content; for a proficiency test, the protocol describing the task for the determination of organochlorine pesticides (OCP) in milk powder should also request the determination of the fat content. On the other hand, the protocol for the certification of OCP in milk powder may only focus on results expressed on the basis of the total milk.

The design of the protocols is largely linked to the objective set to the interlaboratory study. Examples of interlaboratory studies are described in more details in the following sections. It is the task of the organising body and of the pilot laboratory to provide the participants with the adequate (sufficient but not restrictive) information and requirements.

12.3.6. Collection and evaluation of the data

The evaluation of the data generated by the participants in an interlaboratory study, the actions and the conclusions to be drawn by the participants and the organiser or the regulatory body strongly depend on the objective of the study. The way the results are to be reported is also affected by the assigned objective. The results can be evaluated by the organiser and delivered as such to the participants (e.g. tables with raw results). Preferably they may be presented in such a manner that they can be easily interpreted (e.g. with accompanying graphical representations, results of statistical tests) and perhaps be discussed in a meeting with the participants.

12.3.6.1. Collection of results

In order to allow an easy collection of the analytical data the organiser has the task to prepare reporting sheets. These sheets may enclose two types of data sheets.

12.3.6.1.1. Description of the methods

This part is usually not included in reports where a simple proficiency test is foreseen within a larger system of quality assurance or accreditation nor is it for standardisation

purposes. It is an essential element for the evaluation of the performance of a laboratory or of a method with a training objective in so called step by step approach projects (see section 12.7).

Annexes 5.1 and 12.1 give examples of reporting forms elaborated in BCR (Bureau Communautaire de Référence) for the certification of inorganic and organic parameters in environmental matrices. They may be used as a basis to prepare reporting forms for all type of interlaboratory studies. The elaborate questions listed in the forms are intended to remind the participant of important parameters, which affect the quality of the final result. They can also be used in the technical discussion with the other participants when differences in results are noticed. Finally, they form the basic information for the preparation of certification reports in case certification is considered (see chapter 5).

12.3.6.1.2. Reporting the data

The data reported by the laboratories should be comparable and will be treated by the organiser of the study. Therefore, unambiguous information must be available to the organiser to prepare the report of the exercise. This may include tables for raw data, data corrected for dry mass, recovery for organic and organo-metallic determinations, linear response of the detector, calibration range, data expressed on a fat content basis etc. Such results sheets are also illustrated in Annex 12.1.

12.3.6.1.3. Presentation of the data

The organiser should present the data to the participants in the interlaboratory study in such a way that they can extract the answers, which were part of the objective of the study. The participants should have the opportunity to make remarks or corrections. The data should be treated confidentially until the organiser has accepted to disclose them.

12.3.6.2. Technical evaluation

The technical evaluation of the data consists of a scientific inspection of the reports submitted by the participants. In laboratory performance studies, and for a maximum benefit to the participants the organiser should prepare summaries of method descriptions including the data for the most critical steps in the analytical procedure e.g. calibration, pretreatment, extractions and clean-up, separation, final detection. If possible these comparative method descriptions should be presented in form of simple tables where the most important parameters are grouped by item or analytical step. To facilitate the possible discussion in a meeting the data should be presented in a visualised manner (bar-graphs, etc) and the results should be ear-marked to the laboratories. Examples of different types of representations of data extracted from BCR projects (e.g. bar-graphs, Youden-plots, tables etc.) are given in various chapters of this book. In the most ideal situation, a meeting allows participants to discuss the methods. Such meetings are necessary in learning programmes, or in the development of standardised methods. For simple laboratory performance studies the detailed description of the methods is usually not necessary.

The technical evaluation may also lead to the comparison of the results obtained from different methods. It will allow participants to extract information by comparing and possibly discussing their performance and method with other participants applying similar procedures, i.e. it may allow to discover biases in methods. If several enriched materials have been prepared and analysed the organiser may produce Youden plots where trends and systematic errors can appear [10–12]. Such more elaborated data presentations have to be issued with sufficient explanations to avoid misunderstanding and wrong conclusions. More advanced data treatment require the application of suitable robust statistics which have to be carefully chosen to arrive at sound scientific conclusions. Their meaning should always be explained and documented.

12.3.6.3. Statistical evaluation

It has to be emphasised that the statistical analysis of the data of the interlaboratory study cannot explain deviating results nor can it alone give any information on the accuracy of the results. Statistics only treat a population of data and inform on the statistical characteristics of this population. The results of the statistical treatment may raise the discussion on particular data not belonging to the rest of the population. But outlying data can be true and the majority can be wrong. The statistical treatment should be reserved only to those data, which have been produced following the requests laid down in the protocol of the study. In laboratory performance studies or material certification studies it often happens that a majority of participants apply systematically biased methods. An example of such a biased group of data in the determination of a hexachloro dibenzo furan has been published recently [13] and is shown in Figure 12.2. Only one laboratory managed to perform a proper separation of F 118 and F 119 and therefore was likely to be right. A statistical treatment identified the data of this laboratory as an outlying set.

The organiser can apply various statistical treatments to the submitted values [14]. They can be grouped into two categories. The treatment of all individual data or of the mean values of the determinations from each participant. When different methods are applied by the participants, usually, the statistical treatment is based on the mean values of the replicate determinations. Pooling of data can be applied when one method is used on one sample for method performance studies. The statistical evaluation usually involve tests on the homogeneity of the population of individual data or of the mean values e.g. Kolmogorov-Smirnov, Grubbs, Dixon, Nalimov tests and treatment of the variances e.g. ANOVA, Bartlett, Cochran test etc. Within and between laboratory variances should be calculated and compared. Together with the technical evaluation of the results the statistical tests will be the basis for the conclusions to be drawn and the possible decisions to be taken.

12.3.6.4. Conclusions and actions

The conclusion to be drawn and the actions to be taken depend on the initial objectives of the interlaboratory study. They will be discussed in more detail in the following

Lab No	REPLICATES					MEAN	S.D.
01	249	244	218	216	276	241	25
02	190	169	229			195	30
03	176	184	183	188	178	182	5
04	173	172	172	164	170	170	4
06	177	193	151	204	180	181	20
09	185	159	142	149	145	156	17
10	183	181	173	176	182	179	4
11	189	211	197	258	237	218	28
05	107	123	119	119	113	116	6

BAR-GRAPHS FOR LABORATORY MEANS AND ST. DEV.

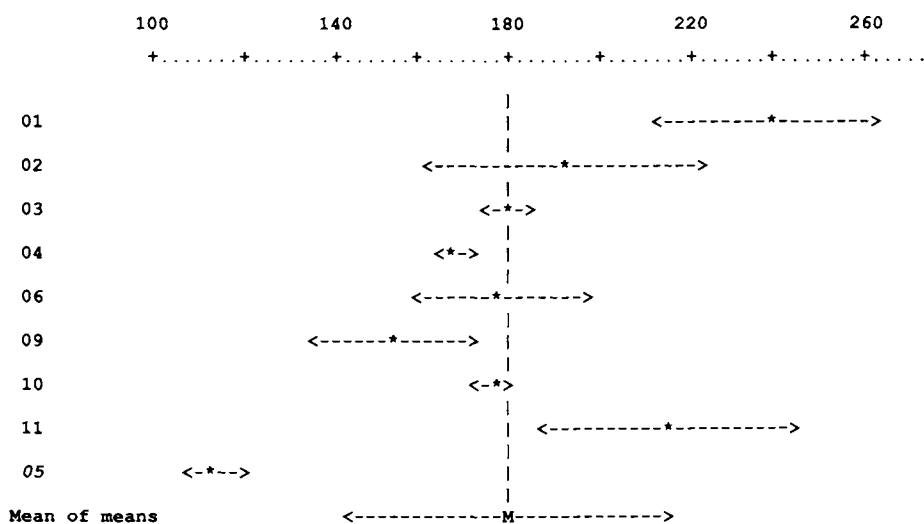


Fig. 12.2. Values for mass fractions of F118 in a fly ash extract reference material, expressed in $\mu\text{g kg}^{-1}$. Results obtained by Lab. 05 correspond to the use of a DB Dioxin column, which allows a better resolution for F118/F119.

sections dealing with the various types of studies. In all cases, it is the duty of the participating laboratory to evaluate its own performance in the study in order to possibly improve.

12.4. METHOD PERFORMANCE STUDIES

12.4.1. Definition

A study in which all laboratories follow the same written protocol and use the same test method to measure a characteristic (usually concentration of an analyte) in order to assess the performance parameters of a method [1].

12.4.2. Fields of application

It is an exercise in which each laboratory uses the same method on a set of identical test samples following the same protocol. Such studies are organised frequently by standardisation bodies e.g. World Health Organisation (WHO), the Food and Agriculture Organisation (FAO), CEN, ISO, etc, national standardisation organisations or professional associations e.g. the International Dairy Federation (IDF), AOAC, the International Federation of Clinical Chemistry (IFCC), etc.. They have the goal to work out and test the performance of (a) method(s) for a well defined parameter and often for a well defined matrix [15]. The study allows to detect sensitive steps in the method description, which may lead to discrepancies in the outcome of the analysis [16,17]. They may also be used to evaluate the ruggedness or robustness of a method and its exact field of application e.g. which type of matrix, what range of concentration of the analyte, possible sources of systematic errors, quality control parameters [18]. The results of the study may also be used to set the performance characteristics of a written standard e.g. limit of detection or determination, between laboratory reproducibility, etc. A method performance study may also indicate whether a written standard developed for the use in various countries, and therefore translated in various languages, has the same meaning and reading in all versions.

A method performance study may also be used to evaluate the performance of a new analytical method or an automated form of an already existing method [1,19]. Under certain circumstances a method performance study may be called intercalibration study. This should only concern exercises where the calibration of methods is investigated e.g. studies on calibrants, calibrant solutions, calibration procedures etc. Such studies are more frequent in physical measurements for metrological purposes.

Investigations on sampling may be conducted as interlaboratory studies. In that case the analysts have to move to one central place with their own sampling equipment. After having taken the sample they perform the measurements in their own laboratory. The influence of the sampling technique on the total uncertainty of the analysis can only be evaluated after having assessed, in a parallel study, the uncertainty due to the final determination.

Such studies are carried out in cases where the sampling technique is of paramount importance, i.e. where the sampling technique is suspect of changing the integrity of the sample but also in cases where it is impossible to produce a common (stable) sample for an analytical intercomparison [20].

12.4.3. Method and protocol

The method performance study focuses on the analytical method. In order to achieve a constructive work the organiser should assure that the method to be used by the participants has been investigated in detail prior to the start of the interlaboratory study. This preliminary investigation should be undertaken by a senior analyst. It should lead to a detailed draft analytical protocol, to a clear definition of the type and number of samples to be distributed and analysed, to indications on the repeatability of the method and its long-term reproducibility. In parallel, a study on the production of proper samples should be undertaken. Additional materials can also be prepared, e.g. calibration tools (pure calibrants or solutions), or blanks with a similar matrix than the test sample, spiked materials, etc.

It may be possible that the method performance study allows a certain degree of freedom in the methods applied. This is a situation encountered when producing new written standard in the field e.g. of environmental monitoring where the analytical state of the art is rapidly changing e.g. dioxin monitoring from incineration plants [21]. In such a situation the minimum requirements on apparatus and procedure have to be specified as well as the degree of freedom left to each laboratory for its own method. The performance criteria set up the basic performance criteria to be achieved. The standard also specifies the way to demonstrate that the set criteria have been achieved (e.g. use of CRMs). This approach allows the laboratories to improve the method when new tools are available e.g. better calibration tools, new phases for chromatographic separations, new detection systems etc. Changes may be introduced in a method only when their performance is at least equivalent to the criteria set in the standard and may allow the testing laboratories to achieve a better performance or more economic and/or rapid determinations.

When sufficient participants are available it is also possible to test on the same materials several methods. Each method should be applied in several laboratories. It may also be of interest to compare the method with an existing method or the non-automated to the automated version of the method.

12.4.4. Participants

Only experienced laboratories with senior analysts should participate in method performance studies. Following the recommendations of IUPAC [2] at least 8 participants should deliver usable results for each test sample and each method or group of methods. When an international standard is used, participants from several countries should be involved.

12.4.5. Materials

As already mentioned one or preferably several test materials should be produced and analysed by the group. W. Horwitz recommends at least five independent materials to properly evaluate the performance of the method. These can be materials of similar

composition and slightly differing analyte concentration (split materials), blind replicate materials or known replicate materials.

12.4.6. Reporting and exploitation of the data

The data should be reported as specified in the protocol with the requested significant figures. Valid data (those free of gross errors and produced following the protocol) should be submitted to various statistical treatment for outlier detection of mean and variance, and an ANOVA treatment to establish the repeatability and reproducibility figures. All these treatments and their sequence are specified in the IUPAC protocol [2]. The final report should contain all individual and statistical data; additional graphical representation e.g. Youden-plots, bar-graphs etc may also be added.

12.4.7. Examples of method performance studies

AOAC has published an impressive list of methods developed through interlaboratory studies. Their procedure follows a strict approach, planning and evaluation of results, following the IUPAC guidelines described above. These methods are now considered as official methods of analysis for many regulatory bodies especially in the food and agriculture field and mainly in USA. The 15th edition of the compendium of AOAC methods has been published in 1990 and contains several hundreds of methods [22]. The US Environmental Protection Agency (EPA) has also used the interlaboratory approach to develop and validate analytical methods for mainly environmental monitoring purposes [23].

The EC-Measurements and Testing Programme (formerly BCR) has also developed over years a broad experience in method performance studies. Two examples of such approaches are given below.

12.4.7.1. Extraction tests for soil and sediment analysis

As described in Chapter 9, the development and use of extraction schemes have started in the early 1980's and aimed to evaluate the metal fractions available to plants (and thus estimate the related phyto-toxic effects) and the environmentally accessible trace metals (e.g. mobility of metals from a soil and potential groundwater contamination) [24–27]. However, the lack of uniformity in the procedures used did not allow the results to be compared worldwide nor the methods to be validated. Indeed, the results obtained are 'operationally-defined' which means that the 'forms' of metals are defined by the determination of extractable elements, using a given procedure and, therefore, the significance of the analytical results is highly dependent on the extraction procedures used. Results are useful and usable only if they correspond to well-defined and accepted procedures. In other terms, the only mean for achieving sound interpretation and basis for decisions is to achieve the comparability of results which automatically passes through an agreement of the procedures to be used, their testing and validation, and their possible implementation as a standard.

12.4.7.1.1. Standardisation

Whereas many discussions arise on the risks that standardisation may 'fossilise' progress in analytical science in a wide range of cases, it is generally accepted that the only way to achieve comparability when using operationally-defined procedures is to standardise these procedures and apply them, using very strict protocols. This does not mean that improvements should not be investigated to ensure progress in the use and resulting interpretation of these schemes; in this case standardisation offers scientists a possibility to speak the same language and decision-makers a way to identify better possible strategies for environmental risk assessment. Extraction tests are widely used for the assessment of the release of inorganic contaminants from soils, sludges and sediments. In many instances, these schemes are included in national (or sometimes regional) regulations. The International Standardisation Organisation (ISO) is coordinating working groups on soil quality (e.g. ISO TC/190) with the aim to identify a range of tests acceptable for possible standardisation. Expert consultations and discussions are based on the selection of existing extraction schemes, e.g. EDTA, DTPA, calcium chloride etc. which should first be accepted as candidate standard tests (on the basis of their scientific significance), then demonstrated to be applicable to various matrices (easiness of use, ruggedness) and possibly tested by expert organisations. This approach requires extensive consultations and interlaboratory testing of the selected candidate standard procedures.

12.4.7.1.2. Pre-normative research on extraction tests

In a first stage, an initial study of the literature and a consultation with European experts were carried out on behalf of BCR [28]. From the variety of schemes published, three single extraction procedures were selected, namely EDTA, acetic acid and ammonium acetate. With respect to sequential extraction, three methods were selected and tested [29] and the results were compared; on the basis of the results obtained, it was concluded that while the agreement between the different procedures was good enough for results to be used in decision-making, there were serious failures in detail which would hamper an accurate comparability of data. Consequently, it was decided to design a simple three-step extraction protocol based on the scheme of Salomons and Förstner [30]. For both single and sequential extraction schemes, interlaboratory studies were designed and conducted with soil and sediment reference materials originating from the Joint Research Centre of Ispra, Italy [29]. The first intercomparison was performed in 1989 with a group of European laboratories.

The results showed that EDTA and acetic acid extraction schemes led to comparable results when the protocols were applied thoroughly; the main sources of error were actually due to calibration errors. However, the ammonium acetate extraction scheme did not result in a good between-laboratory agreement, probably due to the low contents of extracted metals (Table 12.1). The results of this trial was satisfactory enough to communicate them to ISO, along with the EDTA and acetic acid protocols, for discussing their adoption as possible standards. A further interlaboratory study has been performed on a calcareous soil reference material, using EDTA and DTPA extraction procedures [31]; both procedures resulted in a satisfactory agreement between the laboratories which would also enable DTPA to be possibly considered as candidate standard.

TABLE 12.1

MEANS OF LABORATORY MEANS OF EXTRACTABLE CONTENTS OF TRACE ELEMENTS IN SOIL (mg kg⁻¹); ADAPTED FROM REF. [26]

EDTA extracts

Elements	Cd	Cr	Cu	Ni	Pb	Zn
Mean	23.1	8.05	162	16.3	255	492
CV (%)	9.2	25.9	8.5	13.0	11.8	5.8

ACETIC ACID EXTRACTS

Elements	Cd	Cr	Cu	Ni	Pb	Zn
Mean	19.3	26.1	29.2	15.7	3.36	522
CV (%)	7.5	8.2	10.0	18.1	24.6	6.9

AMMONIUM ACETATE EXTRACTS

Elements	Cd	Cr	Cu	Ni	Pb	Zn
Mean	3.43	1.39	5.65	1.42	2.21	18.4
CV (%)	10.9	40.6	23.4	22.5	26.8	22.5

The tests carried out on the sequential extraction scheme illustrated the increased difficulty for achieving comparability of results in comparison to single extractions. Systematic errors were again due to calibration errors but also to a lack of compliance with the written protocols (e.g. differences in shaking time and speed) which highlighted the need to strictly follow the protocol. For only six of the overall means of extraction steps, out of a total of eighteen, the CVs were less than 20%; other values were up to 30% and as high as 80% (e.g. for Pb). The principal cause of poor agreement was certainly lying in the very low contents found (in some cases too close to the detection limits of the techniques used); consequently a second trial was organised in 1992 with a sediment containing higher metal contents [32]. Table 12.2 shows the CVs obtained in the two interlaboratory exercises which demonstrated a clear improvement in the between-laboratory agreement. The three-step sequential extraction scheme was hence considered to be sufficiently robust and valid to yield comparable results. The conclusions of these trials were communicated to ISO for a possible consideration of this scheme as an international standard. It was recognised, however, that work is still needed to test the scheme with different sediment matrices and to adapt it for soil analysis [33].

In the frame of the interlaboratory study on single extraction for soil analysis, an example has demonstrated that it is not sufficient to use a standard protocol to achieve comparability of data, i.e. it is also necessary to strictly comply with the technical requirements of the scheme: one laboratory used a reciprocating shaker instead of the recommended end-over-end shaker and obtained systematically low results; the repetition of the analyses clearly showed that the error was due to this fact (Table 12.3). This

TABLE 12.2

RESULTS OF THE FIRST AND SECOND INTERLABORATORY EXERCISES (STEPS 1, 2 AND 3) ON EXTRACTABLE ELEMENTS FROM SOILS

The table lists the mean of the laboratory means along with the standard deviation (SD) and the coefficient of variation (CV) obtained. the trace metal contents are given in mg.kg⁻¹; adapted from ref. [30]

STEPS	First round-robin			Second round-robin		
	Mean	SD	CV	Mean	SD	CV
Cadmium						
1	7.18	0.81	11.3	0.18	0.02	8.5
2	3.41	0.63	18.5	0.08	0.02	16.9
3	1.03	0.20	20.1	1.02	0.17	16.6
Chromium						
1	1.36	0.20	14.7	1.28	0.41	32.0
2	3.29	1.07	32.5	1.21	0.31	25.6
3	76.3	10.4	13.6	866	126	14.5
Copper						
1	3.69	0.76	20.6	0.23	0.08	34.8
2	3.13	1.96	20.1	0.53	0.33	62.3
3	63.4	13.2	20.8	90.1	7.6	8.4
Nickel						
1	9.76	4.36	44.7	13.0	2.08	16.0
2	5.79	1.54	26.6	1.80	0.23	12.7
3	10.2	3.32	32.5	15.2	2.3	15.2
Lead						
1	5.06	2.50	49.4	0.30	0.17	56.7
2	11.0	8.87	80.6	0.14	0.09	64.3
3	6.93	4.78	69.0	47.7	7.6	15.9
Zinc						
1	262	35.1	13.4	93.9	16.9	18.0
2	140	34.2	24.4	79.7	16.9	21.2
3	89.7	9.14	10.2	676	44	6.5

finding showed that the shaker speed was an important parameter since it represents one of the factors (along with the shaker type) that condition the maintenance of the samples in suspension during the extraction.

12.4.7.1.3. Conclusions

The experience has shown that, while the tests carried out on some schemes resulted in a satisfactory agreement (e.g. EDTA, DTPA, acetic acid), this was not always achieved for other procedures, which demonstrated the inadequacy (or the lack of 'maturity')

TABLE 12.3

RESULTS OF AN INTERLABORATORY TESTING OF A SINGLE EXTRACTION PROCEDURE APPLIED TO A SEWAGE SLUDGE AMENDED SOIL (1N mg kg⁻¹) OBTAINED USING A RECIPROCATING AND END-OVER-END SHAKER, RESPECTIVELY

Shaker	Cd	Cr	Cu	Ni	Pb	Zn
EDTA						
Reciprocating	19.9 ± 1.0	18.2 ± 1.9	165 ± 6	24.3 ± 1.3	149 ± 12	478 ± 15
End-over-end	24.6 ± 1.3	27.5 ± 1.0	218 ± 12	30.4 ± 1.6	232 ± 10	610 ± 29
Acetic acid						
Reciprocating	7.4 ± 0.4	5.5 ± 0.6	17.6 ± 1.0	9.9 ± 0.6	2.37 ± 0.16	257 ± 3
End-over-end	17.5 ± 0.3	17.2 ± 0.2	31.7 ± 0.6	22.2 ± 0.8	1.47 ± 0.16	572 ± 22

TABLE 12.4

RESULTS OF 'WEAK EXTRACTANTS' TESTED IN AN INTERLABORATORY STUDY ON SEWAGE SLUDGE AMENDED SOILS; ADAPTED FROM REF. [32]

A: CALCIUM CHLORIDE EXTRACTABLE CONTENTS (MG/KG)

Element/soil	Mean	S.D.	P	Techniques used
Soil CRM 483				
Cd	0.45	0.05	10	FAAS, ETAAS, ICPAES
Cr	0.35	0.09	9	FAAS, ETAAS, ICPAES
Cu	1.2	0.4	11	FAAS, ETAAS, ICPAES
Ni	1.4	0.2	10	FAAS, ETAAS, ICPAES
Pb	< 0.06	—	8	FAAS, ETAAS, ICPAES
Zn	8.3	0.7	9	FAAS, ETAAS, ICPAES
Soil CRM 484				
Cd	< 0.08	—	9	FAAS, ETAAS, ICPAES
Cr	< 0.09	—	7	FAAS, ETAAS, ICPAES
Cu	0.67	0.29	10	FAAS, ETAAS, ICPAES
Ni	< 0.05	—	9	FAAS, ETAAS, ICPAES
Pb	< 0.06	—	7	FAAS, ETAAS, ICPAES
Zn	0.31	0.17	7	FAAS, ETAAS, ICPAES

p: sets of results (each of 5 replicates)

of the scheme(s) at the time it was tested, e.g. ammonium acetate but also weak extractants such as calcium chloride, sodium nitrate, owing to difficulties in applications (the low extractable contents resulting in a wide spread of results [33]) as illustrated in Table 12.4.

TABLE 12.4

B: INDICATIVE VALUES: SODIUM NITRATE EXTRACTABLE CONTENTS (MG/KG)

Element/soil	Mean	S.D.	P	Techniques used
Soil CRM 483				
Cd	0.08	0.03	6	FAAS, ETAAS, ICPAES
Cr	0.30	0.07	4	FAAS, ETAAS, ICPAES
Cu	0.89	0.22	6	FAAS, ETAAS, ICPAES
Ni	0.65	0.07	5	FAAS, ETAAS, ICPAES
Pb	< 0.03	–	4	FAAS, ETAAS, ICPAES
Zn	2.7	0.8	5	FAAS, ETAAS, ICPAES
Soil CRM 484				
Cd	< 0.05	–	7	FAAS, ETAAS, ICPAES
Cr	< 0.03	–	6	FAAS, ETAAS, ICPAES
Cu	0.48	0.15	8	FAAS, ETAAS, ICPAES
Ni	0.023	0.005	6	FAAS, ETAAS, ICPAES
Pb	< 0.06	–	7	FAAS, ETAAS, ICPAES
Zn	0.09	0.04	6	FAAS, ETAAS, ICPAES

TABLE 12.4

C: INDICATIVE VALUES: AMMONIUM NITRATE EXTRACTABLE CONTENTS (MG/KG)

Element/soil	Mean	S.D.	P	Techniques used
Soil CRM 483				
Cd	0.26	0.05	9	FAAS, ETAAS, ICPAES
Cr	0.27	0.10	8	FAAS, ETAAS, ICPAES
Cu	1.2	0.3	9	FAAS, ETAAS, ICPAES
Ni	1.1	0.3	9	FAAS, ETAAS, ICPAES
Pb	0.020	0.013	4	FAAS, ETAAS, ICPAES
Zn	6.5	0.9	8	FAAS, ETAAS, ICPAES
Soil CRM 484				
Cd	0.003	0.002	7	FAAS, ETAAS, ICPAES
Cr	< 0.06	–	7	FAAS, ETAAS, ICPAES
Cu	1.1	0.4	10	FAAS, ETAAS, ICPAES
Ni	0.033	0.017	6	FAAS, ETAAS, ICPAES
Pb	< 0.06	–	7	FAAS, ETAAS, ICPAES
Zn	0.17	0.05	9	FAAS, ETAAS, ICPAES

p: sets of results (each of 5 replicates)

12.4.7.2. Evaluation of methods for microbiological parameters

12.4.7.2.1. Development of microbiological RMs

Two parallel improvement programmes have been started in 1986 for the development of RMs and possibly CRMs for the quality control of microbiological determinations. The first project focused on target bacteria important for monitoring food of which the CRMs are fully described in chapter 7; the second project dealt with water contaminants. These studies have been described in details elsewhere [35] and are summarised below. The projects had two objectives:

- to test and evaluate the suitability of RM and analytical protocol for the use of the RMs;
- to allow participants from various countries to compare the performance of their methods and help them to improve their quality.

About 50 laboratories participated in each programme. In the course of the successive trials, several microbial strains have been studied and several standard operating procedures have been developed and validated. Each trial was preceded by fundamental research on the stabilisation and the homogenisation of the test strains. In each trial the participants used at least two methods:

- the own home made procedure;
- one common method prepared by the central laboratory.

Additional methods were also tested, in particular when several standardised methods were available. The use of the common methods had two purposes: (1) to allow to test the studied materials with a method best suited for their determination (e.g. test of stability during transport, homogeneity), and (2) to have a reference set of data for the evaluation of the own methods of the laboratories.

The microbial strains studied were *Salmonella thyphimurium* (two exercises), *Listeria monocytogenes* (two exercises) and later *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens* for food microbiology, and *Escherichia coli*, *Enterococcus faecium*, *Enterobacter cloacae* and *Staphylococcus warneri* for water microbiology. The results were discussed in meetings with all participants. Several standard operating procedures common for several methods were developed, tested and amended, e.g. measurement of pH of culture media, measurement of temperature in incubators, statistical treatment of results, etc.

Beside the help to all laboratories to test their own methods of determination, the major outcome of the programmes was the certification of reference materials in spray dried milk powder [36–41] as described in chapters 4, 5 and 7. The microbiology programme also demonstrated that a stepwise approach can be applied in other fields than chemistry even for living organisms.

12.5. LABORATORY PERFORMANCE STUDY

12.5.1. Definition

A study in which laboratories use the method of their choice to measure a characteristic in order to assess the performance of the laboratory or analyst, usually to evaluate or improve performance [1]

12.5.2. Field of application

It is a study, which focuses on the performance of the laboratory and of the analyst. Several examples of such studies are described in literature [42,43]. It helps participants to compare their performance and their method to the performance of other laboratories and/or to an assigned value [44,45]. Repetitive laboratory performance studies to evaluate if laboratories are able to fulfil a given task are often called proficiency testing schemes. First guidelines have been set under the ISO Guide 43 [46]. In 1993, IUPAC has published an 'international harmonised protocol for the proficiency testing of (chemical) analytical laboratories' [3] which sets the basic criteria for the organisation of laboratory performance studies.

12.5.3. Objective and organisation

Laboratory performance studies are organised by private or public entities. Participation can be done on a voluntary basis (e.g. by subscription). It may be mandatory when the laboratory seeks for a special recognition or social status e.g. accreditation. The number of participants may vary from some 20 e.g. for some very specialised measurements such as doping control laboratories of the International Olympic Committee (IOC) to nearly hundred or more, e.g. in the case of the QUASIMEME project described in section 12.5.6.

To have a real impact on the quality of the laboratory performance the participation in interlaboratory performance studies should be done on a regular basis and should be an integral part of the laboratory quality assurance and quality control system. The common project of IUPAC/AOAC/ISO, leading to a harmonised protocol [3] gives several indications and recommendations on the organisation and evaluation of such interlaboratory studies.

The study may concern prescribed (e.g. normalised) methods or the participants may apply their own preferred method. The study involves on a regular basis the distribution of samples to the participants. The frequency of the trials will depend on the type of determination requested, e.g. the difficulty and duration of the determination(s), the frequency of such analyses in the laboratory, the cost/benefit of the test, the duration of the treatment of the data which is influenced mainly by the number of participants and the number of samples to be prepared. Too frequent exercises e.g. more than twice a month, would be inefficient as the laboratory would have no time to evaluate the results between trials. Too long delays between exercises e.g. once a year, does not allow to estimate trends in performance and would be of little help in the laboratories' quality control schemes.

To avoid too long delays between trials the number of participants may be limited. The data should be sent back by the participant in a given short time interval and the outcome of the exercise should be made available as soon as possible together with an advice to poor performers. This allows the participants to take appropriate action in time.

Whatever the frequency and content of the studies will be it has to be clear before

the start and for all participants how the organisation and the final evaluation will look like.

12.5.4. Evaluation of performance

This part of the laboratory performance study is probably the most sensitive to the participants. The responsible of the evaluation may have to draw consequences from the achieved performance for the various participants. To assume this responsibility he needs to be scientifically competent. The organiser should also ensure that the data have been produced without any collusion of participants or falsification. Depending on the number of participants, the objective and the consequences of the study and the frequency of the trials, preservative measures against collusion may be taken (e.g. different and coded sets of test samples, etc). Whatever, it must remain clear to the participants that laboratory performance studies are first of all organised to help them to reach and maintain quality for the benefit of all.

The performance of the laboratories can be evaluated following different rules. It can concern the analytical result compared to the results of the rest of the participants. It can also be a comparison towards an assigned value (i.e. rarely certified reference materials are available and if so they could be easily recognised) and it may also concern reproducibility or repeatability figures. The performance of the laboratories should be appreciated within the given objective of the study, i.e. it is sufficient that the results produced are adequate for the intended purpose of the measurement (e.g. following criteria set by regulations); it is not necessary to appreciate the performance of the laboratory in connection with the absolute possibilities of a method.

In order to bring clarity and confidence in the evaluation of such studies the IUPAC harmonised protocol [3] recommends one evaluation method: the so-called *z*-score. This evaluation is based on the comparison of the obtained results *x*, to an assigned value *X* of a laboratory, which should be the best estimate of the true value. The *z*-score is expressed as:

$$z = (x - X) / \sigma$$

where σ is a target value for the standard deviation. This value can be fixed arbitrarily by the organiser e.g. to allow comparing the performance between similar and successive trials. This is easy for studies on well known parameters with well established methods e.g. standardised methods, deduced from several previous studies. The value of σ can also be deduced from regulatory requirements, which have to be fulfilled by the applied methods. In some circumstances σ cannot be known beforehand and may therefore be a calculation of the interlaboratory standard deviation of the exercise. The selection principle for σ has to be defined beforehand and has to be known by the participants. When a sufficient number of participants are involved the distribution of the results collected in the study will be a Gaussian distribution with *X* as a mean. For such a normal distribution, $|z|$ would have in 95% of the cases a value of 2 or less. A value

over 3 would have a probability of less than 0.3%. As a consequence results of the z-score can be interpreted as follows:

$|z| \leq 2$ satisfactory performance

$2 \leq |z| \leq 3$ questionable performance

$|z| \geq 3$ unsatisfactory performance

Values of $|z|$ over 2 should lead the concerned laboratory to investigate rapidly for the reasons of this weak performance before the next trial of the interlaboratory study starts. An example of z-score is shown in the section 12.5.6. Figures 12.3a–c show graphs of z-scores obtained in proficiency testing schemes carried out within the QUASIMEME (see section 12.5.6) and MICROBATH (bathing water microbiology method development study) frameworks.

Another scoring system also mentioned in the harmonised protocol but not recommended by the authors is the Q scoring system largely used in occupational hygiene monitoring:

$$Q = (x - X)/X$$

where x and X have the same meaning as in the z-score.

It may be interesting or important to obtain repeatability figures from the participants in a study. This may be the case when standardised methods are concerned for which repeatability criteria are specified. Then replicate determinations may be requested. In normal proficiency testing of routine measurements this is usually not necessary.

12.5.5. Relation to accreditation

During the last decade several countries and authorities have installed so called accreditation systems following ISO Guide 25 (soon to become ISO 17025) and EN

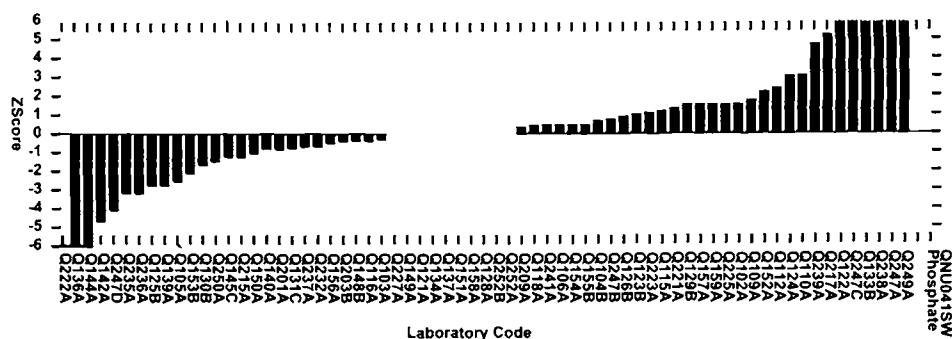


Fig. 12.3a. Z-score presentation for the determination of phosphate in sea water (QUASIMEME project, courtesy of D. Wells, Marine Laboratory, Aberdeen, United Kingdom)

A substantial number of participants did not achieve the $|Z| < 2$ score considered as the minimum performance.

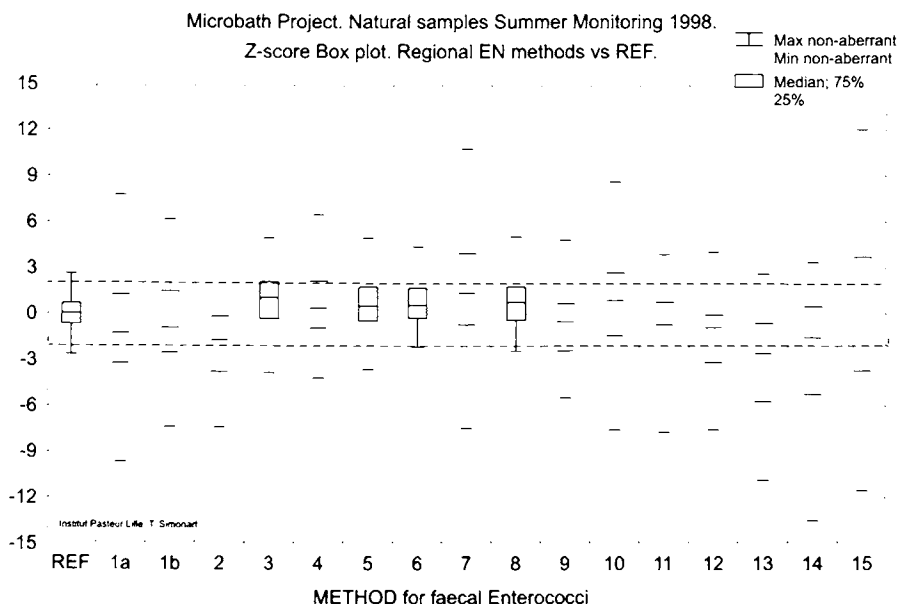


Fig. 12.3b. Representation of Z-scores for evaluating the performance of microbiological methods for faecal enterococci in comparison with a reference method. (SMT, MICROBATH project, courtesy of T. Simonard, Institut Pasteur de Lille, France, 1998).

45001 series of norms [47,48]. Accreditation following these standards consists in organising auditing systems and helping laboratories to comply with a certain number of requirements. Some accreditation bodies have associated to the auditing of a proficiency testing system to verify the performance of the laboratory e.g. NATA — Australia. It is still under discussion in many countries whether results in proficiency testing should be taken into account to maintain the accreditation of a laboratory. As such the EN 45001 standard only recommends participation in interlaboratory studies but also specifies that accreditation cannot be only based on the participation and the results in proficiency tests. The Western European Laboratory Accreditation Co-operation, WELAC now EA, has issued guidelines for the organisation of interlaboratory studies in the frame of accreditation [8]. EA does not state how the results — in terms of z-scores of different trials — should be considered for the accreditation. The level of quality of the organisation of the interlaboratory study and the absence of regulations controlling organisers is one reason for not yet taking action on the basis of results obtained in proficiency tests. Whatever, many analysts are of the opinion that the examination of performance in interlaboratory studies should be an integral part of the auditing and decision for granting accreditation.

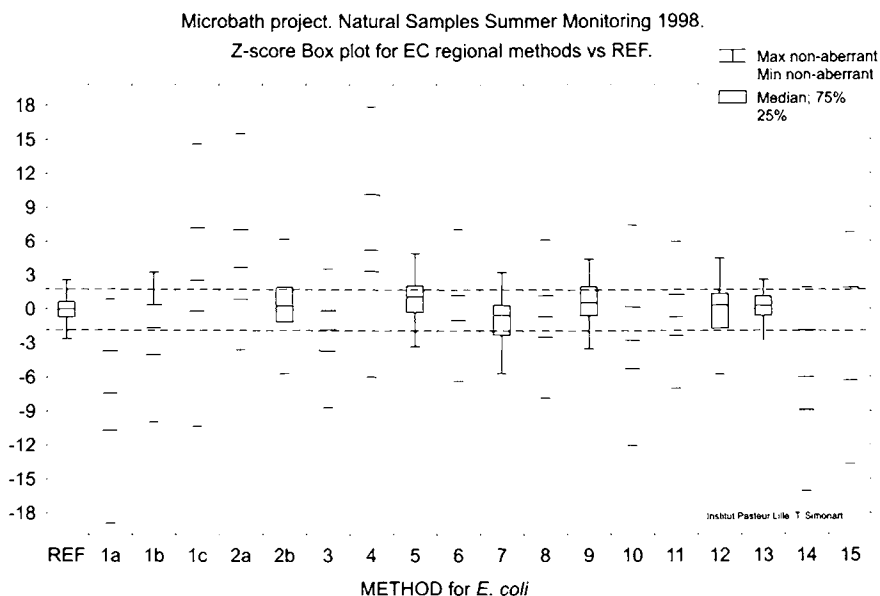


Fig. 12.3c. Representation of Z-scores for evaluating the performance of microbiological methods for *E. coli* in comparison with a reference method. (SMT, MICROBATH project, courtesy of T. Simonard, Institut Pasteur de Lille, France, 1998).

12.5.6. Example of Laboratory Performance Study: Proficiency testing scheme for marine monitoring

12.5.6.1. Introduction

Quality management and laboratory accreditation systems relate to quality activities within a single laboratory and not between institutes, and there is no provision for a comparative assessment of QA data in cooperative marine monitoring programmes. Another element which is absent in existing systems is a structured framework for improvement. Norms and standards are essential to describe, control, and document the quality of information, but these established quality systems do not include an improvement program for the new and developing laboratory, or for the good laboratory which seeks a higher standard of performance. What is missing from these quality systems is a holistic framework, including continuous assessment of methods and/or laboratories, stepwise improvement, and training programs in an international setting. In addition to the laboratory work, the quality control of measurements must be extended to fieldwork, sampling and storage. As a response to the need to establish such a framework, the Measurements and Testing programme has funded a project to help marine monitoring laboratories to set up a continuous quality assessment of routine measurements carried out within monitoring campaigns. This section gives an outline of this project known as QUASIMEME co-ordinated by the Marine Laboratory in

Aberdeen (UK), which has already been described in details elsewhere [49,50]. This project (Quality Assurance of Information in Marine Environmental Monitoring in Europe) has found its place within a regulatory framework [51], namely in support to the Oslo and Paris Commissions (OSPARCOM), the Baltic Monitoring Programme (BMP) of the Helsinki Commission (HELCOM), and the Barcelona Convention in the Mediterranean; it started by examining current guidelines and proposing a structure for the organisation of wide proficiency testing scheme [52] and then developed into a well-structured holistic framework as described below.

12.5.6.2. Framework

The list of chemical determinands initially included in the programme was restricted (Table 12.5) [53].

The participants in the project were mainly laboratories contributing data directly to international marine monitoring programmes. An important feature was to involve people actually performing analytical work in technical meetings and workshops. The project team was composed of the project office staff, a scientific assessment group, national contacts who also acted as a steering group, and, last but not least, the participants.

The overall program started by a circulation of a questionnaire sent to each participant, related to their current QA practices. The responses allowed to evaluate the areas of need and to adapt the improvement schemes [54]. Workshops and seminars were considered to be an important part of the improvement programme since they enabled participants to share expertise and to receive pragmatic and clear advice on how to solve their possible problems.

The project began with a simple series of exercises to evaluate the analytical precision and bias of the participating laboratories. The long-term precision was compared with the documented precision given by the participants in the detailed questionnaire [54].

TABLE 12.5

DETERMINANDS INCLUDED IN THE QUASIMEME PROFICIENCY TESTING SCHEME, ADAPTED FROM REF. [45]

Nutrients	Metals	CBs	OCPs	PAHs
Nitrate	Aluminium	CB28	HCB	Benzo[a]anthracene
Nitrite	Arsenic	CB52	PpDDE	Benzo[a]pyrene
Ammonia	Cadmium	CB101	α HCH	Benzo[b]fluoranthene
Phosphorus	Chromium	CB105	γ HCH	Benzo[e]pyrene
Total-N	Copper	CB118	ppDDD	Benzo[g,h,i]perylene
Total-P	Lead	CB138	ppDDT	Chrysene
	Mercury	CB153	Dieldrin	Fluoranthene
	Nickel	CB156	Trans-nonachlor	Indeno[1,2,3-cd]pyrene
	Zinc	CB180		Phenanthrene
				Pyrene

In many interlaboratory studies, the short-term precision measured within a day or within batch is usually very small. However, when measured over 5–8 weeks, as in this programme, a more realistic and meaningful measure of the precision emerged.

A six-monthly cycle for these exercises enabled to achieve significant improvements by removing obvious and extreme sources of errors (e.g. due to calibration). The key information that came from regular laboratory testing was: the long-term performance of laboratories; the extent and magnitude of specific errors; the improvement made by 'poor performers'; and those determinands and matrices which required specific attention. Participants having a specific problem could join a learning cycle which had a more focused series of exercises designed to overcome the apparent source of error progressively.

12.5.6.3. Test materials

Until recently, most marine biota reference materials have been prepared as a dried powder, which is easier to homogenise, stabilise, store, and distribute. However, this type of RM does not allow checking the sample preparation and extraction procedures. Indeed it is sometimes necessary to use different preliminary procedures for the sample and the RM because of this physical difference. These shortcomings have, in part, been addressed by the stabilised emulsions and homogenised suspensions of marine biota developed by the National Institute for Standards and Technology (NIST) and the National Research Council of Canada (NRCC). However, an holistic laboratory testing and stepwise improvement program requires a succession of RMs which closely matches the specific matrix problems encountered. The programme, therefore, included [49]:

- calibrant solutions of known concentration;
- cleaned-up and raw biota and sediment extracts for organic trace analysis;
- digests from sediments (total HF digestion) and strong acids (partial) for trace metals;
- digests from biota for trace metals; and
- stabilised tissue homogenates for trace metals and for organic trace analysis.

The preparation of each test material followed the same principles as those described in various places of this book with respect to homogenisation, stabilisation, verification of the homogeneity and stability, and establishment of assigned values.

12.5.6.4. Performance evaluation

For the sake of evaluation of laboratory performance, the data assessment procedure had to include extreme values and robust statistics were therefore applied [53]. Z-scores were considered [55] (as described in section 12.5.4, see Figure 12.3) and optimised to fit to the programme requirements [53]; Figures 12.4a and 12.4b show Youden plots of z-scores obtained in the study. This approach enabled to identify various sources of errors, e.g. related to inadequate sample storage conditions, sampling and sample handling (contamination/losses, poor homogenisation, sample degradation, etc.), and lack of analytical quality control.

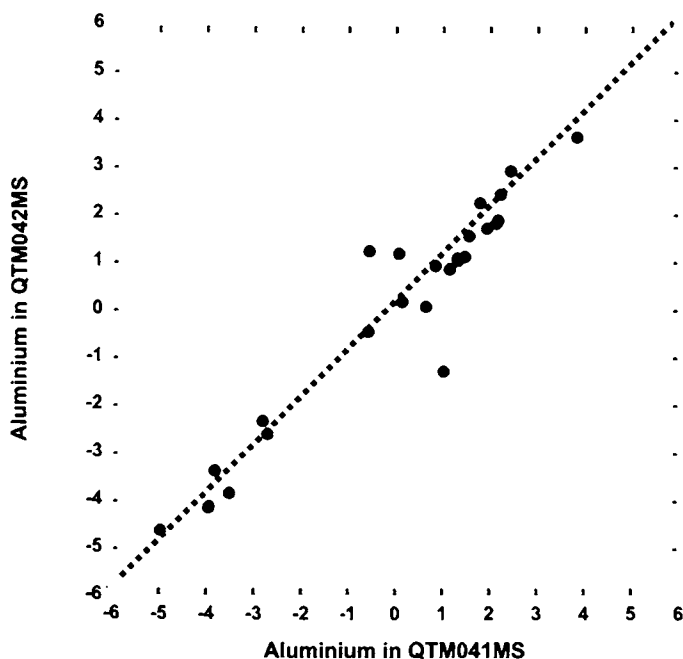


Fig. 12.4a. Youden plot of the determination of Al in marine sediments expressed as z-scores. The plot shows clear systematic differences due to the method of sample digestion. The group of lower (left) results was obtained by digestion methods which did not include any HF treatment e.g. nitric acid or aqua regia. The upper (right) group used an HF treatment or non-destructive methods e.g. XRF.

12.5.6.5. *Developments*

In the first phase of QUASIMEME, the emphasis was to demonstrate that the stepwise approach to improving the between-laboratory agreement can actually work. It was achieved by building up (i) discrete information on the sources of error and how they can be minimised and (ii) the confidence and ability of the participants to actually solve them. This strategy was more successful with a relatively constant group and a small number of steps (3–4). One important aspect was the confidentiality of participants' data and the choice left to them in terms of disclosure their own performance to the International Marine Monitoring Programmes along with their environmental data. QUASIMEME developed into a subscription-type programme which has found a great international recognition and is increasingly expanding [56]. The programme now includes new determinands and matrices, and it is currently complemented by an improvement scheme focusing on sample collection, handling and storage, the QUASH programme funded by the SMT programme [56]. It is expected that both actions will cement the infrastructure for ensuring reliable measurements and data to be provided for a better monitoring of marine quality.

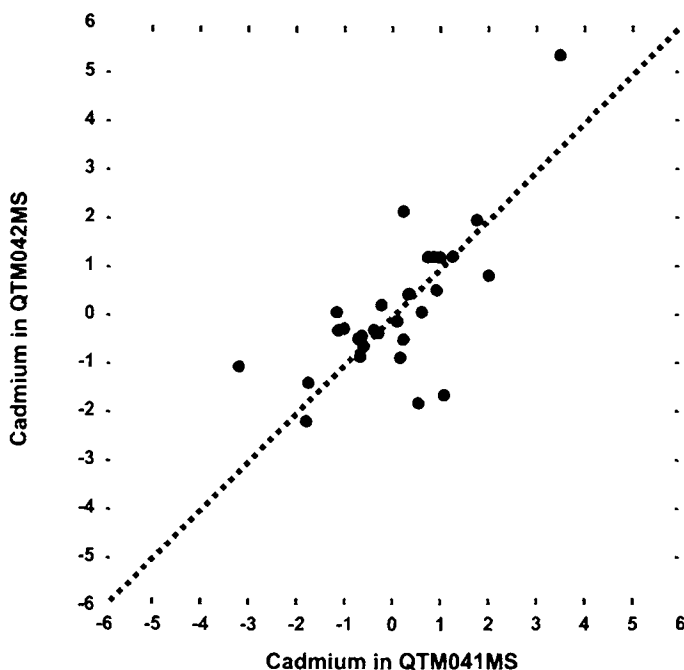


Fig. 12.4b. Youden plot of the determination of Cd in marine sediments, expressed as z-scores. The results show a mixture of both random (data far from the dotted line) and systematic errors. Most data are within $|Z| < 2$.

12.5.7. Conclusion

Participation in interlaboratory performance studies is an essential part of the quality assurance system of a laboratory. It may help to achieve and demonstrate accuracy in the laboratory. Continued participation is necessary to maintain it. Participation in such studies is recommended by accreditation bodies following the EN 45001 norm. Such participation has proven to be of the highest benefit for laboratories [57,58].

12.6. MATERIAL CERTIFICATION STUDIES

The principles of certification studies are described in full detail in Chapters 3, 4 and 5 of this book. Interlaboratory certification studies are organised following the same basic principles as other interlaboratory studies but involve only highly specialised actors. All participants should have demonstrated their quality in prior exercises: degree of accuracy achieved for the determination of the property value to be certified in the given matrix, internal quality control, validation of the method including evidence of statistical control etc. The organiser has also to fulfil many requirements and should be known and recognised for its ability to organise such studies. The best way to establish

the reliability of all participants involved is to ask them to demonstrate their performance in step-by-step improvement schemes as described in section 12.7. This approach has been used by the BCR for all RM where new property values were to be certified for the first time in matrix materials. In all studies, detailed protocols and reporting forms were prepared, of which examples are shown in Annex 5.1 (Chapter 5) and Annex 12.1; these forms request each participant to demonstrate the quality of the measurements performed, in particular the validity of calibration (including calibration of balances, volumetric glassware and other tools of relevance, use of calibrants of adequate purity and known stoichiometry, proper solvents and reagents). The absence of contamination has also to be proven by procedure blanks and chemical reaction yields should be known accurately and demonstrated. All precautions should be taken to avoid losses (e.g. formation of insoluble or volatile compounds, incomplete extraction and clean-up). If the results of entirely independent methods such as IDMS, AAS, voltammetry (between-method bias) for inorganic trace determinations as applied in different laboratories working independently (between-laboratory bias) are in agreement, it can be concluded that the bias of each method is negligible and the mean value of the results is the best approximation of the true value. Numerous examples of such certification studies are given in Chapters 6 to 11.

12.7. IMPROVEMENT SCHEMES

12.7.1. Aims and principles

Improvement schemes can be defined as 'a succession of individual interlaboratory studies in which several laboratories analyse the same test samples for the same characteristics (usually the content of an analyte), following a similar protocol, to validate each individual step of their own analytical method in order to eliminate all sources of systematic errors [5]

Besides the classical interlaboratory studies, improvement schemes enable laboratories to develop and validate all steps of new or existing analytical procedure(s) in adequately organised successive exercises. Improvement schemes may be considered as preliminary studies for laboratory or method performance studies or certification of reference materials [5]. Such programmes are very valuable when the analytical procedures include several complex and critical steps, e.g. for the determination of trace organic compounds or chemical species. They require a long term involvement of the organiser and participants, as well as investment of resources.

12.7.2. Organisation

The organisation of improvement schemes requires a good management capability from the organiser, but also a good scientific background, to design properly the exercises, and evaluate their results. The first step in the organisation of such a programme is to discuss its strategy with all participants involved. This preliminary meeting enables to collect the existing knowledge on e.g. previous studies and available techniques,

and to clearly establish a strategy in relation to the determinands and matrices to be selected. Similarly to other interlaboratory studies, the choice of the determinands and matrices should be based on the feasibility of preparation of homogeneous and stable samples. The improvement scheme should include series of meetings between the different exercises so that the outcome of each evaluation can be discussed with all participants in order to draw consequences and prepare the next exercises. In some cases, additional trials are necessary (e.g. when the results are not sufficiently reliable) which obliges to extend the duration of the programme. All participants should possibly commit themselves at the start of the project to maintain their participation over the entire study. The Figures 2.1a and 2.1b (Chapter 2) illustrate the general principle of a validation which is followed through improvement schemes.

12.7.3. General principles

In chemical analysis, the substances to be determined are rarely directly measurable and sample pretreatment is in most cases necessary to convert or separate the analyte in a form that is compatible with the measurement system. This may imply that the initial physical or chemical composition of the sample is changed without losing control of this change so that the traceability to a determined reference (e.g. fundamental units) is maintained. Typical pretreatment steps are e.g. digestion, extraction, purification; these are frequently followed by intermediate steps such as derivatisation or separation, calibration and final detection. Each action undertaken in one of these steps represents a possible source of error, which adds to the total uncertainty of the final determination.

The objective of improvement schemes is to study and validate each step of the analytical procedure within each laboratory in a collaborative manner. In the best case, each critical step of the procedure should be evaluated in a separate adapted exercise. The individual steps may be studied with a series of different materials. In principle the strategy consists starting from the most simple matrix, e.g. pure solutions and/or mixtures of compounds in solution, to more complex matrices. Such an approach is actually similar to the steps that should be followed when developing and validating a new method in a laboratory.

The difficulty of one particular step may sometimes require subdivision into one or more additional exercises of increasing complexity. In case too many sources of error are identified, it may be necessary to repeat the study on similar (but not on the same) samples. A test sample should never be distributed twice to the participants since pre-knowledge of the material may influence the analyst.

The improvement schemes, usually involving a group of 20 to 50 laboratories, may start with a 'simple' exercise, e.g. by distributing solutions or pure substances. This enables to evaluate the methods of final detection and to possibly optimise them. More complex samples, e.g. complex mixtures of compounds including interferences or extracts, are therefore analysed and the pretreatment/separation steps are assessed; at this stage, the performance of the method is re-evaluated and the procedure may be fully reconsidered if necessary. An intermediate step can be the analysis of a spiked sample, which is followed by real samples. The outcome of the different exercises is discussed

among all participants in technical meetings, in particular to identify random and/or systematic errors in the procedures. When all steps have been successfully evaluated, i.e. all possible sources of systematic errors have been removed and the random errors have been minimised, the methods can be considered as valid.

It should be noted that standardised methods might also be developed and tested by following a similar stepwise approach. In this case, the participants are given less freedom in terms of method development since they are requested to apply a common procedure (e.g. leaching or extraction procedure).

Various examples of improvement schemes organised by the BCR prior to certification campaigns are summarised elsewhere [5] and briefly mentioned in various chapters of this book, e.g. for PCBs and PAHs, organotins, microbiological measurements, etc. The following section describes in detail some of them.

12.7.4. Example of improvement scheme: Methyl mercury in environmental matrices

12.7.4.1. Aim of the project

The justification for an improvement scheme related to methylmercury determination in the environment has been described in Chapters 7 (fish tissue) and 9 (sediment). As mentioned in these chapters, the majority of methods used for MeHg determination consist of an extraction, a separation and an identification/ quantification step. The complexity of the methods and the multiplicity of analytical steps are the reasons why errors are easily made. In view of the need for the improvement of the quality of the analyses, a project for MeHg has been discussed and designed with a group of experts in the frame of the BCR-Programme in 1987. The programme of work was set up in analogy to the successful work of the BCR-group on CBs [59] described in Chapter 7. In particular, the various steps of the analytical methods were studied individually by each of the participants: e.g. extraction, clean-up and separation. This section gives a summary of this interlaboratory programme.

12.7.4.2. The interlaboratory programme

12.7.4.2.1. Analytical methods

Sixteen laboratories from 10 European countries participated in the programme (see chapter 7). The different techniques of separation and final determination used by these laboratories are described in detail elsewhere [60]. Extraction techniques were based on solvent or acid/solvent extraction (e.g. HCl/toluene, H₂SO₄/toluene, toluene/cysteine/toluene). Separation was generally performed by packed column gas chromatography (e.g. 5% DEGS/PS on supelcoport 100–120 mesh, 5% PDEAS on chromosorb W AWD CMS) or capillary gas chromatography (e.g. polar cyanophenylsilicone phase OV-275, methyl-silicone HP-1, CP SIL 8CB). The final determination was made by neutron activation analysis with radiochemical separation (RNAA), electron capture detection (ECD), cold vapor atomic absorption spectrometry (CVAAS) or electrothermal AAS.

12.7.4.2.2. First interlaboratory study

In the first intercomparison three solutions were studied:

- solution 1 contained about 10 mg kg^{-1} of CH_3HgCl in toluene and solution 2 was a mixture of ca. 10 mg kg^{-1} of each of respectively CH_3HgCl , ethyl-HgCl and phenyl-HgCl. These solutions were prepared by the Danish Isotope Centre (DK) by dissolving the mentioned mercury compounds in 5 mL DMSO and in 10 L toluene. Samples were provided in 250 mL bottles protected against light.
- solution 3 was an aqueous solution containing 2 mg kg^{-1} of CH_3HgCl and HgCl_2 respectively. The optimal NaCl and HCl concentrations to avoid adsorption were studied. This sample was prepared in the KFA in Jülich (D).

The stability of the solutions 1 and 2 was verified on the content of 10 bottles stored at 4°C in the dark over a period of 8 weeks at the National Food Agency. Analyses were performed by packed column gas chromatography followed by electron capture detection. Determinations were performed in 3 replicates on each of 3 bottles.

Stability tests of the solution 3 (aqueous solution) were performed at the KFA (Jülich, D) by ion exchange/CVAAS. In a first stage storage experiments at 0°C and at ambient temperature were carried out but no measurable effects of temperature were observed. However, significant losses of Hg were observed after a 100 fold dilution of the solution containing approximately 2 mg L^{-1} of MeHgCl and ionic Hg; therefore it was recommended to dilute the solution only shortly prior to the determination. The stability was verified over 3 months on the content of one bottle and no significant changes were observed for both MeHg and ionic Hg at ambient temperature. All determinations were performed in 7 replicates.

The results of this intercomparison on solutions did not reveal any major discrepancies in the final methods of final determination used. The mean of laboratory means was in all cases very close to the value expected upon preparation ($(10.1 \pm 0.8) \text{ mg kg}^{-1}$ as MeHgCl for solution 1; $(12.7 \pm 1.2) \text{ mg kg}^{-1}$ as MeHgCl for solution 2 and $(2.13 \pm 0.26) \text{ mg kg}^{-1}$ as MeHgCl for solution 3, respectively). Table 12.6 lists the coefficient of variation (CV) obtained between laboratories: both for solutions 1 and 2, the CVs obtained (8.0 and 8.9% respectively) were considered to be acceptable. In the case of the aqueous solution (solution 3), a CV of 12.3% was found to be too high for the present state of the art. On the basis of these results, it was decided to organise a second intercomparison on fish extracts and to repeat an exercise on aqueous solution.

12.7.4.2.3. Second intercomparison

Approximately 4 kg of flounder was purchased at Sletten Havn located in the Niva Bay, 30 km North of Copenhagen, where high levels of total Hg in fish tissues had been reported previously. The fish sample was mixed with redistilled water, homogenised and stored at -20°C . Six subsamples of homogenate each of 0.2 g were analyzed for total Hg by RNAA. The total content was found to be $(191 \pm 20) \text{ mg kg}^{-1}$ (as Hg) on wet mass basis. Extracts were then prepared by the Danish Isotope Centre (DK) and the stability of MeHg was verified by the National Food Agency (DK). Another batch of aqueous solutions as described under first intercomparison was prepared by KFA in Jülich (D) and sent to the participants. The preparation of the samples was the following:

TABLE 12.6

RESULTS OF THE THREE INTERCOMPARISONS INCLUDED IN THE IMPROVEMENT SCHEME ON MeHg IN FISH TISSUES

CV: coefficient of variation (%) between laboratories; range: ratio higher value versus lower value

Solution 1 — First round		Solution 2 — First round		Solution 3 — First round	
CV (%)	Range	CV (%)	Range	CV (%)	Range
8.0	1.3	8.9	1.2	12.3	1.4
Raw extract (second round)		Spiked extract (second round)		Cleaned extract (second round)	Aqueous solution (second round)
CV (%)	Range	CV (%)	Range	CV (%)	Range
16.6	1.6	17.4	1.6	12.5	1.5
8.4	1.3				
Raw extract (third round)		Spiked extract (third round)		Mussel tissue (third round)	Tuna fish (third round)
CV (%)	Range	CV (%)	Range	CV (%)	Range
11.3	1.7	8.8	1.4	17.4	1.7
				13.7	1.6

- *raw extract*: subsamples of 30 g of fish homogenate were mixed with 80 mL HCl and 20 mL CuSO₄. This mixture was shaken, left to react for 15 min and extracted 3 times with toluene to obtain ca. 37 L of extract which was dried by addition of anhydrous NaSO₄ and stored at 5–10°C. The samples were bottled in 500 mL light-protected borosilicate bottles with PTFE gaskets in the screw cap.
- *raw extract spiked with MeHg*: approximately 2500 mL of the raw extract was spiked with CH₃HgCl dissolved in DMSO to obtain a concentration of about 0.011 µg mL⁻¹. Samples were bottled in 250 mL light protected borosilicate bottles.
- *cleaned extract*: subsamples of the raw extract were extracted twice with 150 mL of cysteine acetate. After acidification with HCl, the mixtures were back-extracted twice with toluene. This cleaned extract was dried by addition of anhydrous NaSO₄ and samples were distributed in 100 mL bottles.
- *Aqueous solution*: 1 mg of CH₃HgCl and HgCl₂ respectively were dissolved in water containing 30 g NaCl and 25 mL of HCl per liter. Samples were bottled in 250 mL borosilicate glass bottles with screw caps and stored in the dark at ambient temperature.

All the samples were provided with solid CH₃HgCl calibrants (purity >99.9%). The stability of the extracts stored at 4°C in the dark was verified 5 months after the preparation at the National Food Agency. Analyses were performed by packed column gas chromatography followed by electron capture detection either by direct injection (cleaned extract) or after clean-up with cysteine/toluene (raw and spiked raw extracts) in 3 replicates in each of 3 bottles.

Stability tests of the aqueous solution were performed at the KFA. The stability was verified over 2 months on the content of one bottle (10 replicate analyses) and no significant changes were observed for MeHg.

The results of this intercomparison highlighted that difficulties related to extract analyses were mainly due to a lack of good long-term reproducibility for many laboratories. Capillary GC was found to offer good possibilities but its use was hampered by the absence of commercially available columns. Furthermore, sources of error were likely due to losses of MeHg. A Youden plot (shown in Figure 12.5) of raw and spiked extract demonstrated systematic errors, which was illustrated by the high CVs found between laboratories (Table 12.6: 16.6% and 17.4% for raw and spiked raw extracts, respectively). Better results were obtained for the cleaned extract (12.5%) but the spread was still considered to be too high. However, a consequent improvement was obtained for the aqueous solution analysis (CV of 8.4%).

Owing to the high spread of results obtained for the MeHg determinations in the extracts, it was decided to organise a third round-robin exercise to improve the situation.

12.7.4.2.4. Third intercomparison

A total of 4.6 kg of cod homogenate was prepared as described before from samples fished in the Køge Bay, South from Copenhagen. The total Hg concentration determined by CVAAS in three-fold in the homogenate was ca. $190 \mu\text{g kg}^{-1}$.

- *Raw extract*: portions of 30 g of fish homogenate were treated with 80 mL HCl and 20 mL CuSO_4 solution, shaken and left to react for 15 min. This mixture was extracted three times with about 80 mL of toluene per extraction. Portions of 1500

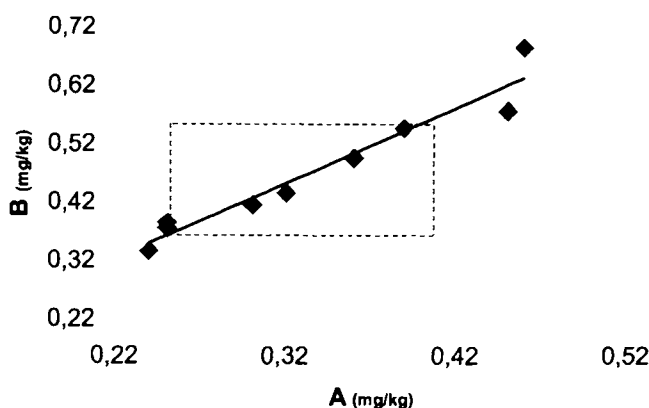


Fig. 12.5. Youden plot of a natural and a MeHg spiked raw extract of fish (adapted from ref.[13]) The results obtained by each laboratory for sample A and B are reported on the horizontal and the vertical axis respectively. The intersection of both results is figured by the points in the graph. Dotted lines of the central square show the standard deviation of the mean of means. In an ideal case all laboratories should have their results in the square. Some sets of results are far from this point which demonstrates the presence of remaining systematic errors in these methods. Results far from the line show random errors.

mL of the toluene extract was then extracted with 400 mL of cysteine acetate solution. After separation, the cysteine acetate solution was acidified and extracted twice with 95 mL of toluene. 4000 mL of toluene was produced.

- *A method and reagent blank solution* was produced alternating with the actual extraction using the same glassware and reagent.
- *Calibrant solution*: MeHgCl was dissolved in toluene in order to obtain a stock solution with a concentration of 44.43 mg kg^{-1} . 10.5 g of this solution was diluted with toluene to obtain a calibrant solution of $266.9 \text{ } \mu\text{g kg}^{-1}$.
- *Spiked extract*: 4.4 g of the stock solution was added to the toluene extract (raw extract) to obtain a concentration in the spiked extract of $(0.9972X + 123.77) \text{ } \mu\text{g kg}^{-1}$ of MeHgCl where X is the concentration in the toluene extract.

The four toluene extracts were bottled in 50 mL light-protected borosilicate bottles with PTFE gaskets in the screw cap. In addition to these samples, mussel and tuna samples were prepared at the Joint Research Centre of the European Commission (Ispra, Italy). The mussel flesh (wet weight) was collected and minced whereas the tuna fish was filleted; the samples were frozen, freeze-dried, ground and homogenised. The two samples were bottled in brown borosilicate bottles each containing 15 to 20 g.

The stability of the calibrant solution and extracts was verified over a period of 5 months at the National Food Agency of Denmark. Analyses were performed by packed column gas chromatography followed by electron capture detection in 5 replicates on each of 3 bottles.

The results of the exercises showed that sources of discrepancies were found for the analysis of the matching calibrant, the most important one being the inadequacy of use of packed chromatographic columns. The CV obtained for raw data was 13.7% (Table 12.6); however, the results improved to 6.3% after outliers had been removed (on technical grounds). The results obtained with CP-SIL 8 capillary columns appeared in most of the cases to be better than the ones using packed columns. It was stated that a precision (as CV) of ca. 3–4% can be achieved with CP-SIL 8 capillary columns lasting for 1–2 years using proper optimisation. An extensive work was carried out to evaluate the CP-SIL 8 columns [61]; the results showed that the use of an on-column insert is recommended to avoid losses of Hg due to contact with hot metal surfaces in the injector. The experiments suggested also that it is important to use capillary columns with a thick film considering that such a film reduces the contact between the volatilised mercury and a possibly not entirely deactivated silica column.

An additional source of error was calibration which should be done systematically using the compound to be determined (e.g. MeHgCl) and not with e.g. HgCl_2 .

The CVs between laboratories (Table 12.6) showed, however, that the state of the art improved in comparison with the second round-robin exercise (11.3% instead of 16.6% for the raw extract, and 8.8% instead of 17.4% for the spiked raw extract).

The mussel and tuna analyses allowed us to test the long term reproducibility of the laboratories. It was noted that interferences were systematically higher with mussel tissue but the higher CV obtained (17.4% in comparison with 13.7% for tuna fish) could also be due to the fact that the MeHg content in mussel was much lower than the one of tuna fish ($(0.14 \pm 0.01) \text{ mg kg}^{-1}$ as MeHgCl in mussel and $(4.33 \pm 0.11) \text{ mg kg}^{-1}$ as MeHgCl in tuna fish).

12.7.5. Example of improvement scheme: seawater microbiology

A project on microbiological determinations in seawater was conducted in a completely different way than those presented above. Seawater samples are very unstable with regards to their microbiological but also for their matrix composition e.g. microflora and microfauna which influence the microorganisms and the analytical methods. In order to evaluate the performance of methods for the determination of faecal bacteria in sea bathing waters, a project was set up in one central laboratory (Institut Pasteur in Lille, France). The same stepwise approach as in chemical projects was applied but instead of circulating samples, the analysts were moved to one central place. Each laboratory used its own method. The organiser in charge provided each of them with all the materials and tools necessary to copy exactly the conditions they encounter at home. This was possible for microbiological determinations as only small laboratory material is used e.g. Petri dishes, glass tubes, filters, filtering devices, incubator, culture media, etc.

A preliminary study with only five laboratories allowed us to set up the organisation and test the statistical treatment of the data. In view of the workload it was decided to split the full group of 34 participants into two sub-groups which worked at different periods. Each sub-group of 17 participants was composed of laboratories from different European regions which are monitoring different seas.

The duration of one step was a full week. Between each step, a meeting to discuss the results and prepare the next steps was organised with both groups together. The various steps of the programme to test the performance of the methods were:

- 1- pure strains of faecal coliforms in sterilised seawater;
- 2- pure strains of faecal streptococci in sterilised seawater;
- 3- pure strains and interfering germs in sterilised seawater;
- 4- real seawater of various levels of contamination and various origin; and
- 5- real seawaters of various levels of contamination and of another origin than in trial 4.

In trial 1 and 2, all participants used their own method and a common method. After trial 1 and 2, the most representative strains for coliforms (*Escherichia coli*) and streptococci (five strains) were selected. As some methods already failed on the artificially contaminated samples of the first two trials, they were not used in trial 3 and 4. After the third trial, three groups of methods for each parameter appeared to be the most reliable. The participants selected these methods as reference methods for the next steps. Each participant applied the three methods on the set of real seawater samples together with the own method. The real seawater samples were transported overnight by plane to the central laboratory. Over the various trials, several participants identified bad practice or weak points in their own method(s) and were allowed to correct for them. Finally, three methods emerged as the most robust, reliable (comparable) and precise for each of the two (groups of) parameters. In total several thousands of determinations on samples of increasing difficulty were performed. The overall conclusions and the results have been published by the Commission [62].

The strategy to perform the work in one central laboratory has shown to be very efficient to study unstable samples and parameters but has two major limitations: (1)

it does not include the environment of each laboratory into the evaluation, which might be a source of unidentified problems, and (2) it cannot be applied for heavy methods such as those usually encountered in chemistry.

The work in one central laboratory could be considered for the study of sampling techniques, in particular for environmental or workplace hygiene problems. Finally, a new project using the same approach allowed to set up a standardised procedure for the validation of microbiological methods, compared to a reference method.

12.8. CONCLUSIONS

Interlaboratory studies play an important role in the field of improvement of quality of chemical trace analysis. They are one of the most flexible and efficient systems to help laboratories to improve and to demonstrate the quality of their work and may allow them to achieve accuracy. They also allow one to verify that a method is able to reach a requested quality and to certify parameters in matrix materials in wide fields of interest. The major limitation of interlaboratory studies is inherent to their organisation. It needs a particular and high scientific and management knowledge. The costs of such studies, when properly conducted, are high; but this cost and the time parameter are accepted by all those who need to evaluate the performance of laboratories or need to develop methods. Therefore, such studies develop more and more, and consequently it was also necessary that international scientific societies such as IUPAC, AOAC International or ISO accept the need to produce guidelines to help organisers to set up proper studies. It may also be necessary very soon to produce minimum requirements on qualification of organisers of such studies, especially if participation becomes mandatory in accreditation where the obtained results may affect the status of the participants.

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INFORMATION SHEET

**CERTIFICATION OF [ELEMENT]
IN [MATRIX]**

LABORATORY:
.....

RESPONSIBLE:

PERSON ATTENDING:
THE MEETING

ELEMENTS/COMPOUNDS DETERMINED:
.....

METHOD(S) USED (IN BRIEF):

Sample intake	Pre-treatment, extraction, derivatization	Calibration	Detection
---------------	---	-------------	-----------

PROJECT
NAME

Interlaboratory study (or certification)

Name of the participant:.....

Laboratory:

PLEASE COMPLETE ALL DATA REQUESTED

Water content (%)

	1	2	3
Sample mass (g)			
% humidity			

Sample intake (g)

1	2	3	4	5
---	---	---	---	---

(if more than five replicates are taken, please complete on an additional form)

CALIBRATION

Reagents used:

- chemical form:
- water of crystallisation/verification of stoichiometry, storage conditions
(describe method):
.....
.....
- dried:
- purity:
- solvent: purity:

in case of a ready made calibrant solution:

- chemical form: in solution
- concentration of final solution:
- verified using:

Description of the method of calibration

* Calibration graph:

- number of points measured: 1 ☐ 2 ☐ 3 ☐ 4 ☐ more ☐
- concentration(s) of measured calibrant solutions:
.....
- matrix matching
reagents used and their purity, concentration in final solution:
.....
.....

* Standard additions:

- number of additions:
- chemical form of element added:
- Amount of element added for each addition:
.....
- additions done before digestion ☐
after digestion ☐

SAMPLE TREATMENT

Sample homogenisation (if prescribed)

- Grinding and sieving ☐
 - Grinder (specify)
 - Particle size (sieve apertures) μm

- Mixing ☐
 - Manual shaking of the bottle ☐
 - Mechanical ☐
 - Other (specify) ☐

- Storage of rehomogenised sample
 - Under Ar ☐
 - In a dried dessicator ☐
 - Silicagel ☐
 - P_2O_5 ☐
 - Other (specify) ☐
 - Light (specify) ☐
 - Temperature $^{\circ}\text{C}$

Chemical pretreatment

- pH adjustment
 - Addition of hydroxide: or acid: to pH=
 - Buffer composition: pH=

- Saponification ☐

- Others (specify): ☐

- Digestion/extraction ☐
 - (if yes, proceed with p.6)

MATRIX DIGESTION

General

Measures taken to avoid contamination (e.g. clean room class ..., clean benches etc.):
.....

Chemical blank:

Acid digestion

- container used (open vessel, reflux app.): ☐
- pressurised ☐
- micro wave ☐

Programme applied (power):

- procedure

	amount of acids (conc.)	purity (vol)	mixing ratio (*)	temperature	duration
step 1
step 2
step 3
step 4
step 5

(*) Please indicate whether the temperature is measured in the heating block or in the digest solution:.....

Alkaline fusion

- procedure

	reagent purity	temperature	duration
step 1
step 2
step 3

- dissolution of the melt
 - solvent:
 - purity :
 - procedure:
.....
.....

Programmed dry ashing

- container used (type, size):
- procedure

	temperature	duration
step 1
step 2
step 3
step 4

 - ashing aids:
 - dissolution of the ash
 - solvent:
 - purity :
 - procedure:
.....
.....

Low temperature ashing oxygen plasma

- container used:
- power applied:
- duration:
 - solvent:
 - purity :
 - procedure:

Combustion

- gas: flow:
- other reagents used: purity:

Methods without digestion

- addition of substance for e.g. sample dilution:
- pressed pellets □
- other treatment (specify):
.....
.....

treatment before final determination

- Sample dilution
 - mass basis ☐
 - volume basis ☐
 - > check of glassware

- sample preconcentration/purification
 - extraction (specifications: see separate form) ☐
 - ion exchange resins ☐
 - type:
 - column:
 - eluent(s):
 - describe method:
.....
.....
 - complexation ☐
 - ligand:
 - describe method:
.....
.....
 - precipitation/filtration ☐
 - describe method:
.....
.....
 - yield determination of those methods ☐

Other (specify):

.....
.....

Addition of an internal standard☐

- type:

added before digestion

☐

added after digestion

☐***Addition of non radio active carriers***☐

(NAA only)

Addition of spike (IDMS)☐

added before digestion

☐

added after digestion

☐

natural abundance of isotopes verified

☐***Addition of other reagents***☐

e.g. for ion strength, buffers:

.....

.....

BLANK DETERMINATIONS

- Cleaning of vessels:

describe method:

.....

.....

- Number of blank determinations (at least one per occasion of analysis):

- Reagents used:

procedure:

.....

.....

DERIVATISATION

Derivatisation procedure

- Hydride generation (NaBH_4) ☐
 Reagent concentration: purity:
- SnCl_2 ☐
 Reagent concentration: purity:
- Ethylation (NaBEt_4) ☐
 Reagent concentration: purity:
- Grignard reagent (specify): ☐

 Reagent concentration: purity:
- Media: addition of ☐
 - CH_3COOH ☐
 - HCl ☐
 - HNO_3 ☐
- Carrier gas: flow: ml/min
- On-line treatment/injection ☐
- Separate derivatisation ☐
- Concentration in final fraction:ng/ml
- Addition of complexing agent: ☐
 Specify:
- Addition of other agent (e.g. anti-foaming): ☐
 Specify:
- Verification of the derivatisation yield: ☐
 describe method:

INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY (ICP-AES)

Plasma and sample introduction

- Nebulizer: type pneumatic ☐ ultrasonic ☐
 composition: glass ☐
 quartz ☐
 PTFE ☐
- Spray chamber: type
- composition: glass ☐
 quartz ☐
 PTFE ☐
- Sample consumption rate: ml/min
- Hydride generation: ☐
 (specifications: see separate form)
- Other pretreatment: specify ☐
- Torch: type
- observation height:.....
- Purge: vacuum ☐ argon ☐ N₂ ☐

Spectrometry

- Spectrometer: simultaneous ☐
 sequential ☐
- Line measured: nm resolution:
- Integration time:
- Background subtraction: ☐
 specify:
- Scanning possible: ☐
- Add if possible spectrum

INDUCTIVELY COUPLED PLASMA / MASS SPECTROMETRY + MASS SPECTROMETRY (ICP-MS and MS)

Plasma and sample introduction

- Nebulizer: type ☐
- composition: glass ☐
- quartz ☐
- PTFE ☐
- Spray chamber: type ☐
- composition: glass ☐
- quartz ☐
- PTFE ☐
- Sample consumption rate: ml/min
- Sample pre-treatment (specify):

Mass spectrometry

- m/e measured: ☐
- Scanning: ☐
- total measuring time:
- mass range:
- Peak hopping: ☐
- measuring time/channel:
- channels/peak:
- Interferences possible (specify): ☐
- Correction: ☐
- Blank value:
- Add if possible spectrum

ATOMIC ABSORPTION SPECTROMETRY (AAS)

Atomisation

Flame ☐Electrothermal ☐Cold vapour ☐Other ☐

- Flame: gas mixture
- Hydride generation ☐
(specifications see separate form)
- Other pretreatment: ☐
- Electrothermal: graphite ☐ pyrocoated ☐
 tube ☐ L'vov platform ☐
 other (specify):
 matrix modifier:
 injection volume:

Programme: Drying Temp Time
 Ashing Temp Time
 Atomisation Temp Time
 Other steps (specify)

Background

correction: Deuterium ☐
 Zeeman ☐
 Smith-Hieftje ☐
 Tungsten-iodide ☐

Absorbance

- Peak height ☐
- Peak area ☐
- Line measured nm
- Add if possible time/absorbance/graph function

ION CHROMATOGRAPHY

IC-Conditions

- Instrument/model:
- Detector type (specify):
.....
- Conductivity detector:
- UV-detection (give wavelengths)
- Others

Pre-column

- Stationary phase:
.....
- Length: cm
- Internal diameter: mm
- Particle size (mesh or μm)

Analytical column

- Stationary phase:
.....
- Length: cm
- Internal diameter: mm
- Particle size (mesh or μm)
- Mobile phase:
- Flow rate: ml/min

Suppressor column

- Stationary phase:
.....
- Length: cm
- Internal diameter: mm
- Particle size (mesh or μm)
- Regeneration time and medium:

ACTIVATION ANALYSIS (INAA, RNAA)

Irradiation

- Thermal neutrons ☐
- Epithermal neutrons ☐
- Fast neutrons ☐
- Photons ☐
- Charged particles ☐
- Nuclear reaction(s):
.....
- Flux:
- Flux gradient correction ☐
- Irradiation time:
- Sample: Size
 dimension
- Calibrants: coirradiated ☐
 separately irradiated ☐
 Flux monitor:
 Composition:
- Shielding: ☐
- Radiochemical separation (specify) ☐
.....
.....
.....

Counting

- Decay time:
- Counting time:
- X-ray counting: ☐
- Gamma-counting: ☐
Geometry
Peak deconvolution

Element/matrix

RESULTS

Nr.	Final digest volume (ml)	Mean total signal	Mean net signal (total - blank)	Conc. in solution (µg/ml)	Blank (µg/kg)	Reproducibility (%)	content in sample (µg/kg)
1							
2							
3							
4							
5							

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